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EFFLUENT DEOXYGENATION AT DIFFERENT TEMPERATURES

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EFFLUENT DEOXYGENATION AT DIFFERENT TEMPERATURES

Final Report

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Division of Research and Training Grants
Federal Water Pollution Control Administration
Department of the Interior

by

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SYNOPSIS

Investigations are reported in the literature in which the effect of temperature on the rate of deoxygenation of raw waste waters was examined. There is evidence to indicate that secondary effluents do not necessarily behave in a similar manner.

The purpose of this study was to determine the relationship between temperature and the rate of both the carbonaceous and the nitrogenous deoxygenation of an effluent from a conventional activated sludge waste water treatment plant. A "jug" dilution technique was used for the biochemical oxygen demand determinations. Numerous nitrite and nitrate-nitrogen analyses were also conducted to establish the kinetics of the nitrogenous phase of deoxygenation as accurately as possible.

The data were employed primarily to calculate the "temperature coefficient" of the modified Arrhenius expression--an expression which relates the monomolecular velocity constant to temperature. The dependence of both the first and second stage ultimate demands on temperature was also investigated. All the final calculated results were then compared with the results of the past investigations reported in the literature.

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REPORT PERIOD

This is the final report of the investigation supported by Grant Number WP-00829 for the period September 1, 1965 to August 31, 1967. The first year of this period was supported by Grant Number WP-00829-01. A progress report covering the period September 1, 1965 to December 15, 1965, was submitted as an Addendum to the application for a renewal grant on December 20, 1965. The second year of this period was supported by Grant Number WP-00829-02.

The principal investigator for the entire investigation was Alphonse E. Zanoni, Associate Professor of Civil Engineering, Department of Civil Engineering, Marquette University.

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I. INTRODUCTION

A well operated secondary or biological waste water treatment plant can accomplish a biochemical oxygen demand (BOD) removal of between 80 and 95 percent and a suspended solids removal of approximately the same magnitude. Depending on the characteristics of the raw waste water, effluents from plants of this type still exert a demand for oxygen when discharged into a receiving body of water. For example, the five day, 20°C BOD of municipal secondary effluents can range from 10 to 50 mg/l. The ultimate or long-term demand for oxygen will be appreciably greater since reduced forms of nitrogen will also be eventually oxidized in addition to the carbonaceous matter. Thus the total amount of oxygen required in the receiving water environment to satisfy the total demand of biologically "treated" effluents must satisfy both the carbonaceous demand still remaining plus the nitrogenous demand. The latter demand can be particularly significant in the case of secondary effluents since waste-water treatment plants are usually more effective in removing the carbonaceous matter.

When a secondary effluent is discharged into a receiving body of water, the organic matter present is used as a source of food and energy by the myriad of microorganisms normally indigenous to such an environment. Provided aerobic conditions are maintained, these microorganisms use oxygen for their metabolic activities and, like all such biological activities, the rate of oxygen uptake or usage is greatly dependent on the temperature of this environment. This rate can be expected to increase with a corresponding increase in temperature, where, in the range of 30 to 40°C, the maximum or "optimum" value is achieved. Increasing the temperature beyond this point results in a very rapid decrease in the rate, reportedly due to the denaturing of certain heat labile proteins, such as enzymes, and the actual destruction of some of the microorganisms. At lower temperatures the deoxygenation activity is greatly reduced to where, near 0°C, it ceases for all practical purposes.

The response of microorganisms to the two extremes in the biological temperature range is not the same, in spite of the fact that the deoxygenation rate is markedly reduced in both cases. Microorganisms held at lower temperatures exist in a form of "suspended animation" and most of them are able to resume their normal activities once they are introduced into a suitable temperature environment. High temperature effects are generally irreversible and, in many cases, lethal.

The relationship between temperature and rate of effluent deoxygenation is of fundamental importance to the sanitary engineer. This knowledge is essential in accurately appraising the effects of discharging a secondary waste water treatment plant effluent on the dissolved oxygen of a receiving body of water. The current rapid rate of urbanization and industrialization is intensifying the water quality control problem to the point where in the not too distant future nearly one hundred percent of the sewered population in this country will be served by treatment facilities of one type or another. It is also very likely that the trend will be towards the requirement of biological or secondary treatment in many areas. The Federal Water Quality Act of 1965 will, according to present indications, also have a very profound influence on accelerating the above trend to higher degrees of treatment.

The degree to which a secondary effluent discharge will affect the minimum or critical dissolved oxygen level in a receiving body of water is intimately associated with the temperature of the effluent and of the receiving water at the time of discharge. Thus a knowledge of the kinetics of both carbonaceous and nitrogenous effluent deoxygenation for the range of temperatures normally occurring in the environment, is essential for calculations of this type. In the northern part of the country, temperatures of receiving waters can easily vary from 0 to 30°C and even higher.

Knowledge of this type is also of economic importance in cases where the extent of secondary waste water treatment facilities is based on the available assimilatory capacity of a receiving body of water. This assimilatory capacity will vary markedly with the seasons of the year, or in other words, with temperature.

Extended aeration treatment and stabilization ponds involve biological activities quite similar to those occurring during secondary effluent deoxygenation. Also, these operations are of current interest since they involve the production of nutrients, principally the nitrate and phosphate ions, with their associated fresh water fertilization problems. Temperature will, therefore, have an important influence on the behavior of these ions, and the design and operation of these facilities can only be efficiently accomplished if a complete knowledge of the extent of this influence were available.

II. SCOPE OF THE INVESTIGATION

The major purpose of this investigation was to examine the effects of temperature on the rate of deoxygenation of a conventional activated sludge waste water treatment plant effluent. This study was concerned with the long-term deoxygenation behavior, that is, the deoxygenation progression was followed until both the carbonaceous and nitrogenous phases were, for all practical purposes, completed. In an effort to minimize any variations in results attributable to varying substrate characteristics, a minimum of three different test runs, each at different temperatures, were set up on one day to form a test series. During the later stages of the investigation when more incubators became available, five test runs were set up in each series.

Because of the nature of the test substrate, special consideration was given to the nitrogenous phase of the deoxygenation. This was accomplished by conducting numerous nitrogen analyses in addition to the overall oxygen usage measurements. It was then possible through the use of these nitrogen data to delineate the two distinct deoxygenation phases and thus facilitate the subsequent mathematical analyses.

A "jug" dilution technique was employed to incubate the effluent at the various temperatures. Because this technique has not been widely employed, numerous ancillary studies were conducted to insure its accuracy and reliability. Primary attention was directed to reaeration and seed correction procedures.

During the final stages of this investigation, special studies were set up to establish the possible mechanisms which might govern the nitrogenous phase of effluent deoxygenation. In these studies seed quantity was varied and ammonia nitrogen was added to the diluted sample in an effort to gain a clearer understanding of this activity.

III. THEORETICAL AND HISTORICAL CONSIDERATIONS

A. Deoxygenation of Secondary Effluents

For the purposes of this investigation it was assumed that the deoxygenation progression of a diluted secondary effluent can be adequately described by the well-known monomolecular (sometimes referred to as unimolecular or first order) biochemical oxygen demand equation. The biochemical oxygen demand (BOD) of a waste water, treatment plant effluent, river water or similar type of material refers to the amount of oxygen required to stabilize, under continuous aerobic conditions, the organic matter present, considered over a certain specified period of time and at a specified temperature. Generally the unqualified term, BOD, refers to the standard value or the amount of oxygen required over a period of 5 days and at a temperature of 20°C. Within the context of this investigation, where various incubation temperatures and periods were employed, the term has the same general meaning as deoxygenation or simply, oxygen demand.

The BOD expression commonly employed is the one which dates back to the classical work in 1925 of Streeter and Phelps[1] and followed in 1927 by Theriault[2] and is presented in the integrated form as follows:

$$y = L_c(1 - 10^{-k_ct}) \text{ - - - - - - - - - - - - - - - - - - - } (1)$$

where, y = oxygen used in (t) days or BOD expressed in milligrams per liter (mg/l)

L_c = ultimate carbonaceous or first stage demand (mg/l)

k_c = carbonaceous velocity constant expressed in days^{-1}

t = time in days

The above equation describes only the first stage or the carbonaceous phase of the total deoxygenation. At the time when the first stage is nearing completion, a second phase commences and proceeds at a rate independent of the carbonaceous deoxygenation and has also been traditionally described by a monomolecular equation as follows:

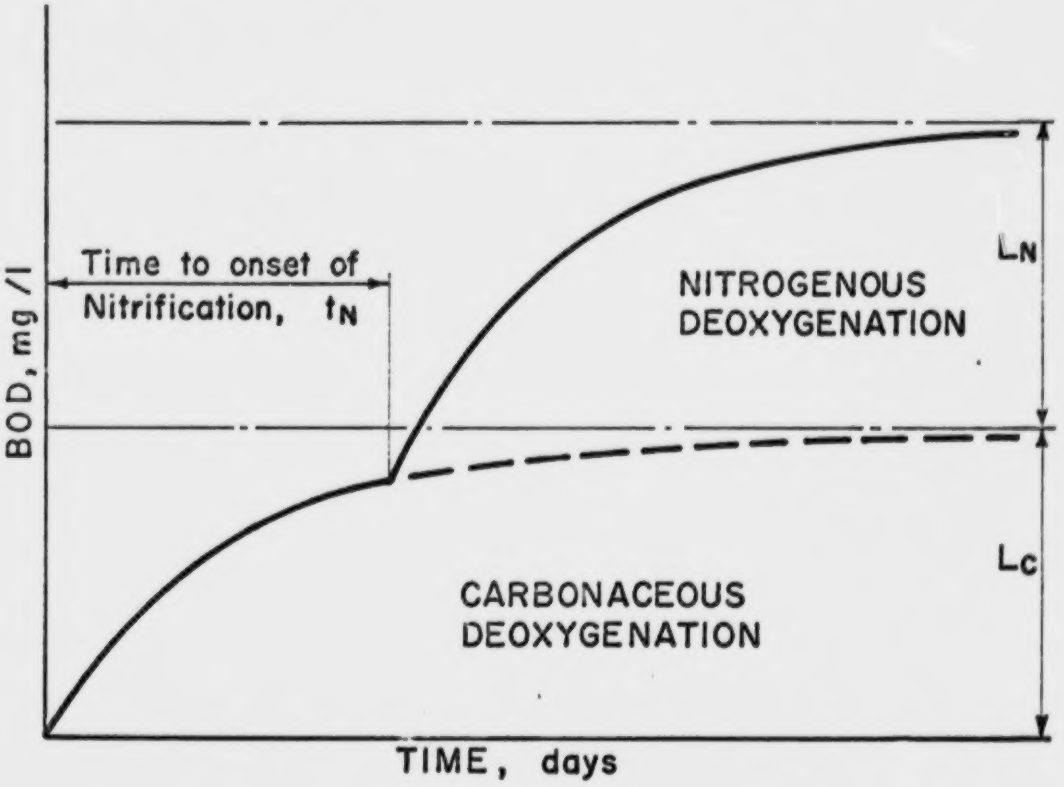
$$y = L_N(1 - 10^{-k_N(t - t_N)}) \text{ - - - - - - - - - - - - - - - - } (2)$$

where the terms have the same meaning as described previously except that the subscript, N, is used to identify the nitrification stage and, t_N, is the time lag in days to the onset of nitrogenous deoxygenation. Thus the total BOD at any time can be described by a summation of the two expressions as follows:

$$y = L_c(1 - 10^{-k_c t}) + L_N(1 - 10^{-k_N(t - t_N)}) \text{ ------- } (3)$$

Figure 1 presents an "idealized" sketch of the monomolecular deoxygenation curve of a secondary effluent showing the two separate stages. The term "idealized" is used because it is not always possible to discern clearly when the first stage ends and the second begins. Such factors as characteristics of the substrate, quantity, viability and type-distribution of microbial population, can result in a BOD progression where it is difficult to differentiate the two separate phases -- in fact, there is a strong evidence that the two stages can proceed simultaneously from the start of incubation. It is this situation which made it necessary for the inclusion of numerous nitrogen analyses for the accurate mathematical interpretation of the complete deoxygenation progression. This situation will be covered in greater detail later in the report when actual laboratory results are presented.

Though both deoxygenation stages represent an exertion of oxygen demand, the microbial activity involved in the two cases is substantially different. Carbonaceous deoxygenation is brought about by a group of microorganisms referred to as heterotrophs, or more appropriately, chemoorganotrophs, in which organic



(1) General Equation of Carbonaceous Deoxygenation

$$\underset{\text{(Dead organic matter)}}{C_X H_Y O_Z} + O_2 \xrightarrow[\text{Microorganisms}]{\text{Saprophytic}} CO_2 + H_2O$$

(2) General Equation of Nitrogenous Deoxygenation

$$2NH_3 + 3O_2 \xrightarrow[\text{Bacteria}]{\text{Nitrite forming}} 2NO_2^- + 2H^+ + 2H_2O$$

$$2NO_2^- + O_2 \xrightarrow[\text{Bacteria}]{\text{Nitrate forming}} 2NO_3^-$$

General Form of BOD Curve

FIGURE 1

compounds serve as the oxidizable substrate and the important end products are carbon dioxide and water. The microorganisms which are involved in nitrogenous oxidation, on the other hand, are referred to as autotrophs, or more appropriately, chemolithotrophs, in which carbon dioxide is the sole or the principal form of carbon, and energy is derived through the oxidation of an inorganic substrate, namely ammonia and the nitrite ion. The ammonia is oxidized to the nitrate ion in a series of two distinct or principal steps as follows:

$$2NH_3 + 3O_2 \xrightarrow[\text{bacteria}]{\text{nitrite forming}} 2NO_2^- + 2H^+ + 2H_2O \quad ---- (4)$$

$$2NO_2^- + O_2 \xrightarrow[\text{bacteria}]{\text{nitrate forming}} 2NO_3^- \quad ------------ (5)$$

As noted in the first equation, the first principal end product of the nitrification process is the nitrite ion. The organisms which are capable of bringing about this transformation belong to the genera Nitrosomonas and Nitrosococcus. The nitrite to nitrate transformation depicted in the second equation is primarily the work of the genus Nitrobacter. Though these genera are felt to be the most important ones involved in these transformations, there are others that have been reported from time to time in the literature. Excellent reviews of this interesting biochemical transformation with the properties and physiology of the organisms involved are available in the literature.[3,4,5]

Another important distinction between the carbonaceous and nitrogenous forms of deoxygenation is the type of dissolved oxygen depletion brought about by each in a receiving body of water. The dissolved oxygen used in the exertion of the former represents a total liability since it has been irreversibly reduced in the form of water molecules. In the nitrification process, however, the dissolved oxygen has been converted into a chemically combined state in the form of the nitrate ion. Under a suppressed dissolved oxygen level or under complete anaerobiosis, this combined form of oxygen can serve as an electron acceptor in the metabolic oxidation-reduction reactions in place of dissolved oxygen. This microbial operation is referred to as denitrification. However chemically combined oxygen cannot be used by the macro-live forms, such as fish, for their respiration needs. This whole concept was aptly discussed by Phelps[6] in the context of the pollution and natural recovery of a stream. "What has been said . . . regarding the stream pollution effects, indicates the importance of distinguishing these two stages as far as possible. The BOD of the carbonaceous stage . . . represents a stream liability. It requires oxygen if nuisance is to be avoided. That of the nitrogenous stage will draw upon the dissolved oxygen resources of the stream also, but since nitrate oxygen is available as an oxygen source, nitrification in the stream in no way depletes total oxygen resources. In fact, by lowering the total dissolved oxygen it stimulates reaeration and actually increases total oxygen assets."

B. Temperature Effects on the Deoxygenation Kinetics of Secondary Effluents

The earliest reported work on the effect of temperature on the deoxygenation of waste water or polluted water was done in 1910 by Pleissner,[7] but Streeter and Phelps[1] were the first some fifteen years later to present an empirical relationship between the velocity constant (k) and the incubation temperature as follows:

$$\frac{k_1}{k_2} = \theta^{(T_1 - T_2)} \text{ - } (6)$$

where k_1 and k_2 are the velocity constants at temperatures T_1 and T_2°C respectively and (θ) is a constant for a specific temperature range referred to as the "temperature coefficient." They found, on the basis of experimental results using both waste water and river water, the average value of θ to be 1.047 for the temperature range 10 to 30°C. Later Theriault[2] demonstrated the similarity between equation (6) and the well-known Arrhenius equation for chemical reactions and for this reason this equation is often referred to as simply the Arrhenius relationship in the literature. Other investigations conducted through the years, mostly in an attempt to more accurately define the θ value over the entire range of biologically significant temperatures, have been reported by Greenfield et al.,[8,9] Moore,[10] Gotaas,[11] Schroepfer et al,[12,13] Stoltenberg and Sobel[14] and most recently, Zanoni.[15] A complete review of this subject plus a study of the deoxygenation kinetics of a raw municipal waste water at temperatures ranging from 2 to 40°C has also been prepared by Zanoni.[16]

One cannot intelligently compare the results of the above investigations without a knowledge of the many details involved. This fact is demonstrated in Table 1 which presents a summary of some of the pertinent details of past investigations. As noted, numerous factors can influence the finally established θ values, among which can be included temperature range, type of substrate, methods of chemical analyses and procedure for the evaluation of velocity constants. Two other points are also apparent upon an examination of this summary. First, very little work has been done on secondary treatment plant effluents. The available evidence indicates that the behavior of this material is markedly different from that of the raw waste waters. Yet there is a reported evidence that θ values based on raw waste waters have been used in applications where secondary effluents were involved. It is apparent that such a situation can result in erroneous conclusions.

Second, the effect of temperature on the nitrification phase of deoxygenation has almost been totally ignored. To the writer's knowledge there has not been any θ values reported for this particular activity. Yet in most practical applications one must consider the effects of the nitrogenous deoxygenation at different temperatures, particularly when secondary effluents are involved.

TABLE 1

Summary of Investigation Involving the Effect of Temperature on Deoxygenation Rate

Investigations	Year	Temperatures or Temperature Range Employed (°C)	θ	Test Substrates
Pleissner[7,20,37]	1910	7.20 and 37	(a)	river water
Streeter & Phelps[1]	1925	10-37.5	1.047(b)	Average value for river water and domestic sewage
Greenfield et al[8,9]	1926	2-20	(a)	domestic sewage
Theriault[2](c)	1927	9-20	1.053	river water
		20-30	1.049	
Moore[10](c)	1941	0.5-5	1.145	domestic sewage
		5-20	1.065	"
		0.5-20	1.026	river water
Gotaas[11](e)	1948	5-15	1.109	domestic sewage
		15-30	1.042	
		30-40	0.967	
Schroepfer[12,13] (f)	1960 & 1964	4-20	1.135	Average values for conventional activated sludge process effluent and river water(g)
		20-30	1.056	
Soltenberg & Sobel[14](h)	1965	5-30	(a)	river estuary water
Zanoni[15,16](j)	1966	2-15	1.126	domestic sewage
		15-32	1.047	
		32-40	0.985	

(a) Test results were not used to evaluate a θ value.

TABLE 1

Continued

(b) Arithmetic average of the results of three different investigations employing varying analytical procedures.

(c) On the basis of his own data and all the previous work done up to that time concluded a reasonable θ value to be 1.047 for the temperature range 2-30°C for both domestic sewage and river waters.

(d) Actual test run temperatures were 0.5, 5, 7.5, 10, 18 and 20°C; employed both phosphate and bicarbonate dilution waters; evaluated nitrification velocity constants but did not calculate the corresponding θ values.

(e) Actual test run temperatures were 5, 10, 12, 15, 20, 25, 20, 34, 35 and 40°C; employed both phosphate and bicarbonate dilution waters and in some series varying amounts of sea water were also added; evaluated nitrification velocity constants but did not calculate the corresponding θ values.

(f) Actual test run temperatures were 4, 20 and 30°C.

(g) Average results were very close for both types of substrate.

(h) Actual test run temperatures were 5, 10, 15, 20, 25 and 30°C.

(j) Actual test run temperatures were 2, 5, 10, 15, 20, 25, 30, 35 and 40°C; different θ values were obtained for both the short-term and long-term deoxygenation data; investigation confined to solely the carbonaceous stage.

IV. GENERAL TEST PROCEDURE

A. Test Substrate

The test substrate used for the entire investigation was the final effluent from the Milwaukee Sewerage Commission, conventional activated sludge waste water treatment plant, located on the Jones Island along the shore of Lake Michigan. The nominal capacity of the plant is 200 mgd. The raw waste water flow is first passed through coarse bar screens, followed by grit chambers and then through rotating fine screen drums. Following the juncture where the screened waste water is mixed with the return activated sludge, the flow is split with about 60% of the total directed to the newer East plant and the remaining 40% directed to the original West plant. Both plants provide essentially the same type of treatment, approximately six hours of ridge and furrow type aeration followed by approximately two hours of clarification. Following final clarification, the effluent flows in a common channel to an outfall located on the lake shoreline. The waste sludge is thickened, vacuum filtered, dried, screened, bagged, and finally sold under the well-known brand name, "Milorganite."

Effluent samples used for this study were obtained from the main East plant effluent channel. A continuous flow of this effluent is pumped to the plant operator's quarters in the East plant and used routinely by the operating personnel to obtain samples for analysis. Approximately 8 gallons of grab sample were obtained from this line on the day the particular test series was set up. Though actual laboratory data on the effluent samples used will be included later in the report, a general idea of the characteristics of the test substrate employed can be obtained by examining some of the pertinent 1966 operating data for the East plant presented in Table 2.

B. Biochemical Oxygen Demand (BOD) Determination

The biochemical oxygen demand (BOD) test was employed throughout this investigation to follow the deoxygenation or oxygen demand progression of the effluent with time. Basically the BOD analysis was conducted in accordance with the recommendation of Standard Methods.[17] Because of the nature of the test, some revisions in the procedure were felt to be advantageous. The revisions pertain to the normality of sodium thiosulfate used in the DO analyses, method of seed correction, and dilution procedure. The reasons for these changes plus the supporting evidence of ancillary studies which warranted them are discussed below.

1. Dissolved Oxygen (DO) Determination

The Alsterberg (azide) modification of the Winkler method was employed for all the dissolved oxygen (DO) determinations except that 0.01N sodium thiosulfate solution was used instead of 0.025N as recommended. Studies

TABLE 2

Average 1966 Operating Data for Milwaukee Sewage Commission Waste-Water Treatment East Plant

Total Plant Sewage Flow (mgd)	186.3
Total East Plant Sewage Flow (mgd)	108.9
Detention time in Aeration Tanks (hours)	6.7
Air Quantity (cu.ft. per gal)	1.2
Suspended Solids	
Raw Screened Sewage (mg/l)	274
Final Effluent (mg/l)	14
Percent Removal (%)	95
BOD	
Raw Screened Sewage (mg/l)	293
Final Effluent (mg/l)	15.4
Percent Removal (%)	94.5
Total Nigrogen as N - Raw Screened Sewage (mg/l)	32.4
Effluent Nitrogen Analysis	
Total Nitrogen as N (mg/l)	11.0
Nitrite - Nitrogen	0.20
Nitrate - Nitrogen	0.11
Chloride (mg/l)	153

conducted by the writer[16] and others comparing the accuracy of DO determinations using 0.005N, 0.01N and 0.025N sodium thiosulfate solutions indicated that the highest degree of accuracy is obtained with the 0.01N solution.

Since there are so many variables which affect the BOD determinations, it was felt that any additional accuracy in measuring the DO would be helpful. Thus all the DO values throughout the course of this investigation were obtained by titrating 203 ml of the diluted effluent sample with a 0.01N solution of sodium thiosulfate.

2. Dilution Procedure

Based on the writer's past experiences with the BOD analysis, the decision was made almost at the outset of this investigation to employ a so-called "jug" dilution technique for all the deoxygenation measurements instead of the conventional BOD dilution procedure. This procedure was felt to be more practical for this particular study mainly because an unlimited quantity of incubated sample was always available to conduct the various necessary nitrogen analyses. Also, because it is a well-established fact that the nitrification phase of deoxygenation can be quite erratic, it was felt that the dissolved oxygen analyses and the nitrogen analyses should be conducted on the same incubated sample rather than from a large number of smaller individually incubated bottles. Such a procedure results in obtaining a much more consistent upward or downward progression of data. Erratic values become suspect at the time the laboratory data are being obtained, providing opportunities for duplication of analyses when deemed necessary by the analyst. Only through the use of this technique was it felt possible to obtain a reliable oxygen depletion balance for the two different types of deoxygenation over the entire length of the test run. Finally, the jug procedure had the added advantage that a single dilution can be employed throughout the entire period of incubation. This is not possible with the conventional dilution procedure thus introducing the possibility of an additional source of error.

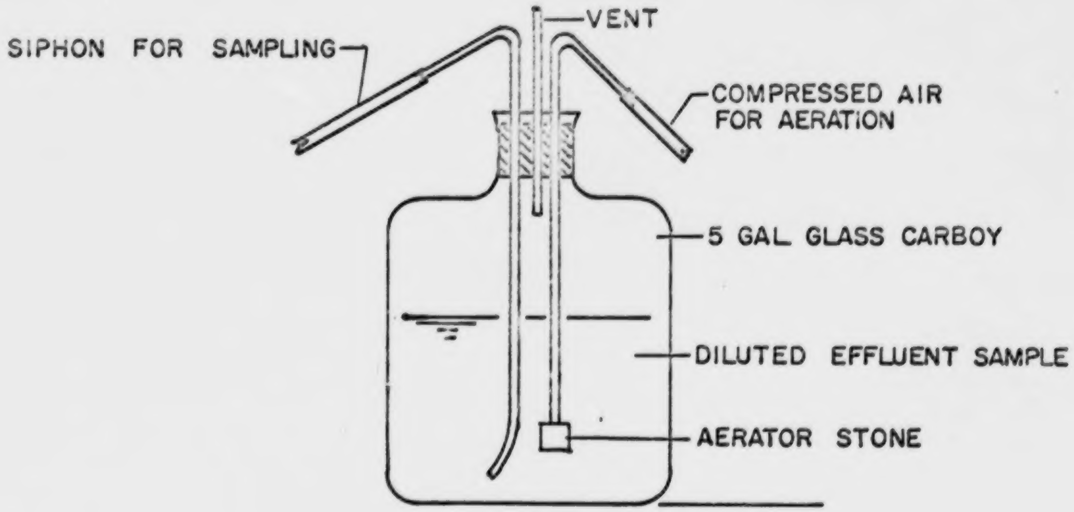
This matter of whether or not varying sample dilutions will influence the magnitude of the BOD values and the resulting kinetics of the deoxygenation progression is worth noting at this time. Reports in the literature are somewhat conflicting. Ruchhoft et al[18] found that the use of high dilutions in setting up BOD's of raw waste water will tend to produce erroneously high results. Much of their data, however, were based on results in which very small oxygen depletions had occurred. They attributed the error to the erratic behavior of nitrification at higher dilutions. Gannon[19] stated that dilution had a significant influence on the routine BOD results of a river water, particularly in the usual dilution ranges of 25-100%. The BOD magnitudes involved were only in the ranges of approximately 2-7 mg/l. Schroepfer et al[12] on the other hand, working with the activated sludge plant effluents found that the varying dilutions had a negligible effect on the BOD results. The writer's[16] conclusions after conducting numerous BOD analyses on domestic sewage concur with those of the latter investigators. It would appear, from the information available, that the greatest degree of error would be most likely to occur when very high dilutions are employed, when sizable DO depletions (greater than 2.0 mg/l) do not occur in the diluted sample bottle, or when well stabilized substrates in the lower BOD ranges are being analyzed, and finally, when active nitrification is occurring. Apart from the above, however, it would seem that the most ideal situation would be the one where the same results can be obtained with the fewest number of dilutions.

Various modifications of the "jug" or "pooling" technique have been described by Elmore,[20] Orford et al,[21] and Krittrell and Kochtitsky.[22] The one employed in this investigation closely follows the one of Elmore.[20] None of the techniques could be used directly as described in the literature because of the peculiarities or specific problems of this investigation; namely, very long incubation periods, incubation temperatures ranging from 5 to 35°C, the need for the use of a well acclimated seed, and finally, the requirement of conducting the various nitrogen analyses for the same incubated sample.

The actual procedure employed was as follows. Five gallon glass carboys were used as the "jugs." Rubber stopper assemblies containing glass tubes for siphoning samples, aerating and venting were constructed for the carboys as shown in the sketch presented in Figure 2. On the day the test run was set up, accurately measured volumes of effluent sample and standard BOD dilution water were added to the carboy. The dilution water was well acclimated to the temperature of the test run and completely saturated with oxygen prior to use. The effluent was quickly adjusted to the test run temperature and thoroughly aerated. In all of the test runs the amount of effluent dilution was the same. A total of 18 liters of diluted sample were set up, 3.60 liters of which was the effluent sample and 14.40 liters, dilution water, resulting in exactly a 20 percent dilution of the effluent. Next, a well acclimated effluent seed was added to the diluted sample according to a procedure that will be described in the following section. Finally, a clean magnetic stirring rod was added to the carboy and left in it through the entire duration of the test run. The entire contents were again aerated to the saturation condition and then stirred gently with a magnetic stirrer. Following

FIGURE 2

JUG APPARATUS FOR SAMPLE INCUBATION AND REAERATION

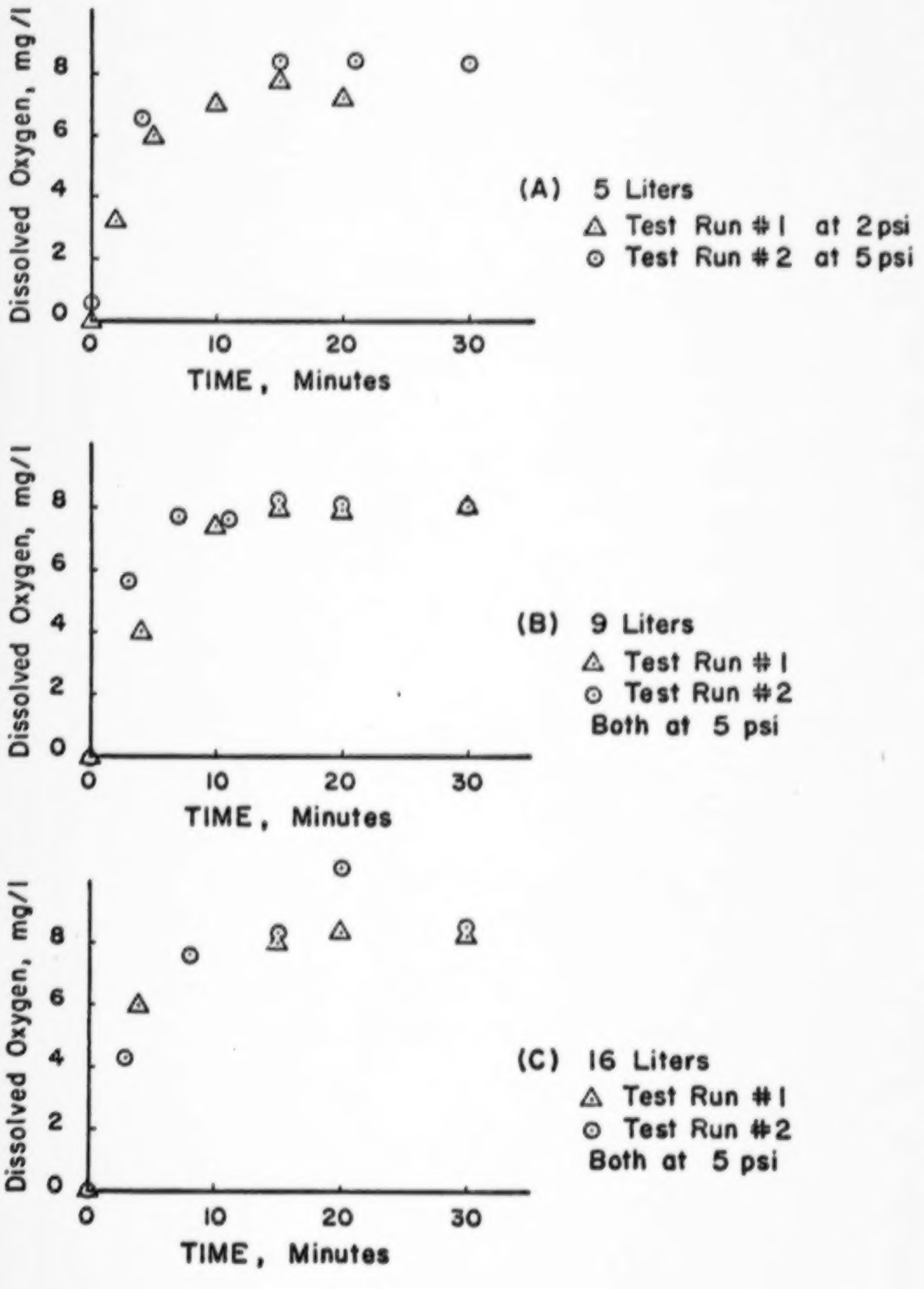

VENT
SIPHON FOR SAMPLING
COMPRESSED AIR FOR AERATION
5 GAL GLASS CARBOY
DILUTED EFFLUENT SAMPLE
AERATOR STONE

saturation the aeration was terminated and after approximately 10 minutes the diluted effluent was then siphoned into approximately a dozen individual BOD bottles while the contents were being continually stirred. At least two of the bottles were used to determine the initial dissolved oxygen value and the others, after filling the wells and sealing the tops with plastic food wrap and rubber bands, were placed in the incubator. The carboy was tightly sealed with a rubber stopper and also incubated at the test run temperature. After an appropriate time of incubation, the dissolved oxygen in one of the sealed BOD bottles was determined. Nitrogen analyses were always conducted with diluted sample taken directly from the carboy following vigorous agitation. When the dissolved oxygen level in the sealed bottles approached anywhere from 2 to 4 mg/l, the remaining BOD bottles were emptied into the large carboy and the contents again reaerated and stirred. Following saturation a new batch of BOD bottles were filled and the test run was continued as described above.

This procedure necessitated that the carboy be removed from the constant temperature incubator for periods of at least one-half hour or longer for the reaeration operation. Such time periods could cause variations in the temperature of the diluted sample, particularly at the very high and low temperature test runs. To minimize these fluctuations a bench-type insulated incubator was designed and constructed. The incubator was constructed out of two layers of 3/4-inch plywood with an intermediate layer of insulated material. The incubator was constructed such that a magnetic stirrer could be slipped into its base. An opening just large enough for the top of the stirrer unit was cut out of the bottom. When the incubator was closed only the neck of the carboy protruded from the incubator box. Therefore, the aeration, siphoning and stirring operations were readily accomplished while the carboy was completely insulated from the laboratory environment resulting in an insignificant change in the temperature of the jug contents.

One question which had to be answered in the above procedure was the length of aeration time required to bring the contents of the carboy to oxygen saturation conditions following a period of deoxygenation. A series of tests were set up to establish this value. Tap water was added to a 19 liter carboy and the dissolved oxygen was reduced to zero using sodium sulfite plus a small amount of cobalt chloride as a catalyst. By trial and error it was found the 75 mg/l of sodium sulfite and 5 mg/l of cobalt chloride was required to depress the dissolved oxygen to just about zero when the tap water was at room temperature, or 23°C. Since the quantity of water in the carboy would also influence the aeration time; to check this test runs were conducted with 5, 9, and 16 liters of tap water. The gage pressure of the air in the line leading to the aeration stone was also varied to ascertain whether or not this factor was also critical. The results of all of the test runs are presented in Figure 3. As noted, 15 minutes of aeration appeared to be more than adequate under all conditions to achieve full oxygen saturation of the water in the carboy, at least at incubation temperatures of 23°C and above. At lower incubation temperatures, slightly longer time periods may possibly be required. These data served as the basis for aerating the contents in the jug whenever the reaeration operation was necessary.

It was felt best to use the same percent effluent dilution in the carboy for all the test runs regardless of the incubation temperature and the actual characteristics of the effluent on the particular day of the test run. From the preliminary deoxygenation tests and an examination of the Milwaukee East plant operation data, it was estimated that the sum of the ultimate carbonaceous and nitrogenous demands could range from 30 to 80 mg/1, with the most likely value around 60 mg/1. The use of undiluted effluent directly or a very low dilution of it would be impractical considering the numerous reaerations which would have to be accomplished throughout the BOD progression.



TEMPERATURE = 23°C Constant

Time of DO Saturation for Different Volumes

FIGURE 3

Yet a very high dilution would also be undesirable because of the expected lower sensitivity of the laboratory analyses, particularly at the early incubation periods. Special considerations had to be given in this regard to the optimum accuracy of various nitrogen analyses. A realistic compromise appeared to be an effluent dilution of 20 percent. From the standpoint of oxygen demand of the diluted effluent in the carboy this would mean a total ultimate value from 6 to 16 mg/1. Assuming an average depletion of approximately 5 mg/1 of dissolved oxygen prior to reaeration, this would mean anywhere from 1 to 4 reaeration operations would be required for each of the test runs--a value felt to be both reasonable and practical.

One last point which should be considered regarding the jug dilution is how closely the BOD results of this procedure compares with those of the conventional dilution method. Elmore,[20] in his original description of the method, presented laboratory data to demonstrate the high degree of precision possible with the technique when comparing five replicate, 30-day duration, deoxygenation test runs using a river water and reaerating four times during the duration of the test runs. He also conducted other tests to demonstrate that the reaeration operation itself did not materially affect the BOD results. Gannon[19] results were conflicting in this regard. Using a river water sample and a secondary effluent he obtained a "good comparison between the reaeration and the standard dilution procedures." However when comparing the results using a secondary effluent from an industry and a river water sample, the dilution method gave consistently higher results over the reaeration procedure. The oxygen demands of the latter substrate were very low, which means that they were very highly stabilized and thus, more likely to show a disparity between the two procedures. At any rate, because of the nature of this investigation; namely, long term test runs, numerous incubation temperatures, need for large quantities of incubated sample for nitrogen analyses, etc., it was felt that the reaeration procedure still offered the best promise for the most complete fulfillment of all the requirements of this investigation.

3. Seed Correction

One of the most important factors which had to be considered in an investigation of this type was that of the use of a seed. It is a recommended practice to use an acclimated viable seed whenever the BOD or other similar biochemical-type analyses are conducted. The seed insures the presence of a myriad of microscopic forms of live at the onset of the incubation period which is essential for the inception and continuation of the biochemical reaction.

Since the seed material itself exerts a demand for oxygen, an appropriate correction for this oxygen utilization must be made in the calculation of the net deoxygenation attributed to the diluted sample. Because of the range of incubation temperatures used in the investigation, the need for a well acclimated and viable seed was particularly significant.

Three different methods were considered for the use of a seed and the determination of its corresponding correction. The first method considered was to use a separate jug and sealed BOD bottles for just the seed alone in addition to the set-up required for the diluted sample. Such an arrangement was felt to be undesirable from a practical standpoint merely because of the limited incubator space. More important, it was felt that the resulting seed correction would more likely be quite erratic, and often subject to question as the greatest percentage of the deoxygenation occurs in the nitrification phase. The work of Ruchhoft and his co-workers,[18] as pointed out previously, demonstrated that the deoxygenation during this phase was inherently erratic. This situation has also been experienced by the writer in his previous studies.

The second method considered was to eliminate the use of a seed entirely. The assumption here is that the effluent sample itself would provide the necessary seed. This procedure would work well if the test run incubation temperature was that of the temperature of the effluent when it was obtained. At higher and lower incubation temperatures, however, an extended lag develops at the outset of deoxygenation. In the analysis of the data for determining the deoxygenation constants, it is then necessary to discard those values considered to be influenced by the lag. Past experience with raw sewage as the test substrate has proven this procedure to be quite difficult and can easily lead to erroneous conclusions. It is felt that this difficulty will be compounded in the case of secondary effluents because of the exceeding low BOD values during the initial stages of deoxygenation.

The last method considered and investigated was to add the seed in as concentrated form as possible, containing primarily viable microbial forms of life, and excluding dissolved organic matter that would exert an oxygen demand. It was felt that one way by which this could be accomplished was by first passing settled supernatent from a well acclimated effluent seed through several layers of gauze to remove any debris. This filtrate would then be passed through a membrane filter.* The material remaining on the filter was considered to be primarily viable microorganisms. This residue was then rinsed thoroughly with sterile buffered dilution water to wash out from the residue, as much as possible, the soluble organic material exerting an oxygen demand. The filter plus the residue was then deposited into the jug containing the diluted sample. The assumption made was that the demand exerted by the seed added in this manner would be so low as to be considered negligible. Also, even if some demand was exerted, it would be about the same percentage of the entire demand for all the incubation temperatures thus reducing its significance in the final comparative results. Thus, the desired purpose of having a viable seed at the outset of the incubation without a need for seed correction would be achieved.

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*Millipore Filter Corp., Cat. No. HAWG 04700

A number of ancillary tests were set up using a seed concentrated in the manner above to demonstrate whether or not this scheme would function effectively. To simplify the studies a standard glucose-glutamic acid solution as described in Standard Methods[17] was employed as the test substrate in all of the test runs. In each test run a series of 5-day BOD analyses were set up using an acclimated effluent seed in the conventional manner, that is, the settled seed was added directly to the dilution water. Another series of BOD analyses were set up exactly in the same way except that the settled seed was first concentrated on a membrane filter and the filter was added to the dilution water. The amount of seed added in both cases was equivalent to 5 ml of seed per liter of dilution water.

A summary of the results are presented in Table 3. This summary represents the average of numerous individual analyses; good enough to insure that the variations in the results were attributed to the different manner in which the seed was employed rather than to the inherent variations in the determination itself. Some slight modifications of the procedure between the various test runs are described briefly in the remarks column. The actual difference in the magnitude of the BOD from test run to test run is not of concern, since it may be due to the variations in the strength of the test substrate. What is important is the difference in the magnitude of the BOD in each test run, as this indicates a difference in the viability of the seed.

A review of this table indicates that adding the seed concentrated on a membrane filter generally was not as effective in exerting a BOD as adding the seed directly. Such was the case in test runs 1, 3 and 4. In spite of the fact that both seeds were just as effective in test run 2, the overall results were such that using a seed concentrated in the manner described appeared not to be as effective, at least within a period of five days, as the seed added in the conventional way.

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TABLE 3

BOD of Glucose-Glutamic Acid Using Seed Concentrated by Membrane Filter

Test Run	Seed Addition	Average 5-Day BOD (mg/l)	Range of 5-Day BOD (mg/l)	Remarks
1	Conventional	242	240-244	
1	Membrane Filter	141	134-166	Seed pass thru gauze mat prior to concentration on membrane filter.
2	Conventional	144	141-147	
2	Membrane Filter	146	143-151	Agitated membrane filter violently in jug before using dilution water.
3	Conventional	206	198-216	Seed pass thru gauze before use.
3	Conventional	194	192-199	Seed was not passed thru gauze before use.
3	Membrane Filter	144	138-150	Seed passed thru gauze before concentration on membrane filter.
4	Conventional	181	172-194	
4	Membrane Filter	137	133-152	Seed plus membrane filter incubated in jug for two days before use.

In addition to the BOD analyses conducted above, numerous microbial plate counts were also conducted on the dilution water seeded by the two procedures described. These results were not conclusive primarily because of the relatively high microbial counts obtained in the incubated unseeded dilution waters. These counts had the effect of masking variations in counts which might be attributed only to the different manner in which the seeds were added. Also, the forms of microorganisms which would develop in a well acclimated secondary effluent are not necessarily the types which would develop rapidly on the rich glucose tryptone extract agar medium employed for the counts.

The desirability of adding a concentrated seed in order to eliminate the need for a seed correction warranted further studies to develop a successful procedure. The possibility of concentration by centrifugation was then investigated. An ancillary investigation was devised to try this possibility. Well acclimated effluent seed after passage through gauze was centrifuged in 15 ml. tubes at approximately 3000 RPM (top speed for the centrifuge available in the laboratory) for 15 minutes. After centrifugation, approximately 13 ml of the supernatant was carefully pipetted from the tube and discarded. Thus a seed concentration of about 7.5 volumes to 1 volume was obtained. The subnatant, or the concentrated seed, was added directly to the dilution water. In terms of the original volume of seed, an equivalent of about 5 ml of seed was added to each liter of the dilution water. This seeded dilution water was used to set up the 5-day BOD of the glucose-glutamic acid solution. This set-up was compared with one using a dilution water seeded in the conventional manner. The results of the two different test runs are shown in Table 4. From these results it appears that the concentrated seed was as effective as the conventional or normal seed in exerting the 5-day BOD of the glucose-glutamic acid. The DO depletion due to the seed alone was negligible in both of the test runs. The use of the concentrated seed, however, indicates that less oxygen demand exerting materials are being introduced with the seed. Thus it was felt that it would not be necessary to apply a correction to the total BOD data. Also, because the amount of seed added in the concentrated form amounted to less than one ml per liter of diluted sample, any correction to the nitrogen analyses for material added in the seed would also not be practical.

It was decided to employ a seed concentrated as described above for all of the test runs reported in this investigation. Effluent from the Milwaukee Sewerage Commission activated sludge plant was used for the seed. The seed was incubated at the test run temperature for one

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TABLE 4

BOD of Glucose-Glutamic Acid Using Seed Concentrated by Centrifugation

Test Run	Seed Addition	Average 5-Day BOD (mg/l)*	Range of 5-Day BOD (mg/l)*	5-Day D.O. Depletion due to Seed Alone (mg/l)
1	Conventional	223	216-228	0.07
1	Concentrated	224	216-228	0.02
2	Conventional	228	223-235	0.02
2	Concentrated	216	209-224	0.0

*Average of five replicate samples

to two weeks prior to its use--the longer time periods were employed for the lower temperature test runs. On the day of the test run the settled effluent was passed through several layers of gauze prior to centrifugation. After centrifugation the seed was added directly to the diluted effluent in the 5 gallon carboy. None of the test runs exhibited any noticeable lag as a result of adding the seed in the manner described above.

C. Other Analytical Procedures

Numerous nitrogen analyses were conducted throughout the course of this investigation in addition to the BOD determinations. The principal nitrogen analyses were nitrite-nitrogen and nitrate-nitrogen. Total kjeldahl nitrogens were also run but usually only at the beginning of the test runs. Ammonia-nitrogen determinations were also conducted during two special test runs set up in the final stages of the investigation. Chloride determinations were also made, primarily because the chloride concentration is needed for the nitrate-nitrogen determination. For the most part, all of these analyses were conducted in accordance with the recommendations of *Standard Methods*.[17] However, some revisions were felt to be necessary, primarily in the nitrate-nitrogen determination, as will be discussed below.

1. Nitrate-Nitrogen

Of the methods presented in Standard Methods[17] the phenol-disulfonic acid method was felt to be the most useful for this investigation since it can be used for nitrate-nitrogen levels as low as 0.01 mg/l. The brucine method was tried but it was felt to be unsatisfactory because it is not accurate below the range of 1 mg/l and the tedious procedure does not lend itself to numerous, random-type analyses. The major problem with the former method as stated in Standard Methods[17] is that it, ". . . is subject to severe chloride interference, necessitating complete chloride removal for a correct result." The recommended method for removing chloride is to precipitate it out with a sivler sulfate solution. However, according to Sawyer,[23] this technique is difficult to accomplish and could lead to erroneous results, since excess silver ions themselves can interfer with the colorimetric procedure. Even in spite of this problem, chloride removal would be tedious and impractical because of the large number of analyses which have to be run. An ancillary study was set up to examine the extent of the interference which would occur for the expected chloride concentrations. It was found that the interference due to the chlorides could be corrected empirically knowing the chloride level involved. This study with the results obtained are discussed in full detail in the following section.

Another problem with the phenoldisulfonic acid test is that the nitrite ion responds like the nitrate ion and thus tends to produce an erroneously high reading. Nitrite-nitrogen concentrations in excess of 0.2 mg/1 only can cause a problem and Standard Methods[17] recommends the oxidation of nitrite to nitrate with either potassium permanganate or hydrogen peroxide when this level is present in the sample. This oxidation procedure never seemed to work successfully when attempted during the course of this study and thus the practice was discontinued. In the final analysis of the data nitrate data which were obtained when the nitrite level was greater than 0.2 mg/1 was automatically excluded from the calculations. Fortunately, because of the original 20 percent dilution of the effluent, this combination of nitrite and nitrate-nitrogen occurred rather infrequently.

All transmittance readings were made on a Model 14, Coleman Universal Spectrophotometer using matched 13 mm square cuvettes.

a. Ancillary Study on Chloride Interference

It has been well established by Taras[24] and Chamot et al[25] that the presence of chlorides decreases the formation of yellow-colored alkali salt of nitrophenoldisulfonic acid. According to Sawyer,[23] chlorides interfere because of their reducing action. Under acid conditions HCl gas is evolved. But some chlorides act to reduce nitrates before they can escape from solution as shown below:

$$Cl^- + H^+ \rightarrow HCl\uparrow$$

$$6Cl^- + 2NO_3^- + 8H^+ \rightarrow 3Cl_2\uparrow + 2NO\uparrow + 4H_2O$$

In this study an attempt has been made to obtain the actual NO_3-N concentrations from the observed concentrations of NO_3-N and chlorides, without removing chlorides from the sample prior to its analysis by the standard phenoldisulfonic acid method. This eliminated the tedius process of removing chlorides from the sample with silver sulfate and possible error due to the excess silver ions added. Standard curves as shown in Figure 4 were prepared for NO_3-N concentrations of 0.1 to 0.6 mg/l by following in general the procedure outlined in Standard Methods[17] with the following exceptions.

(1) The standard nitrate solution was prepared merely by diluting the stock nitrate solution.

(2) Two blanks were run each time and were processed in the same way as the standard samples, that is, 100 ml were evaporated in the alkaline

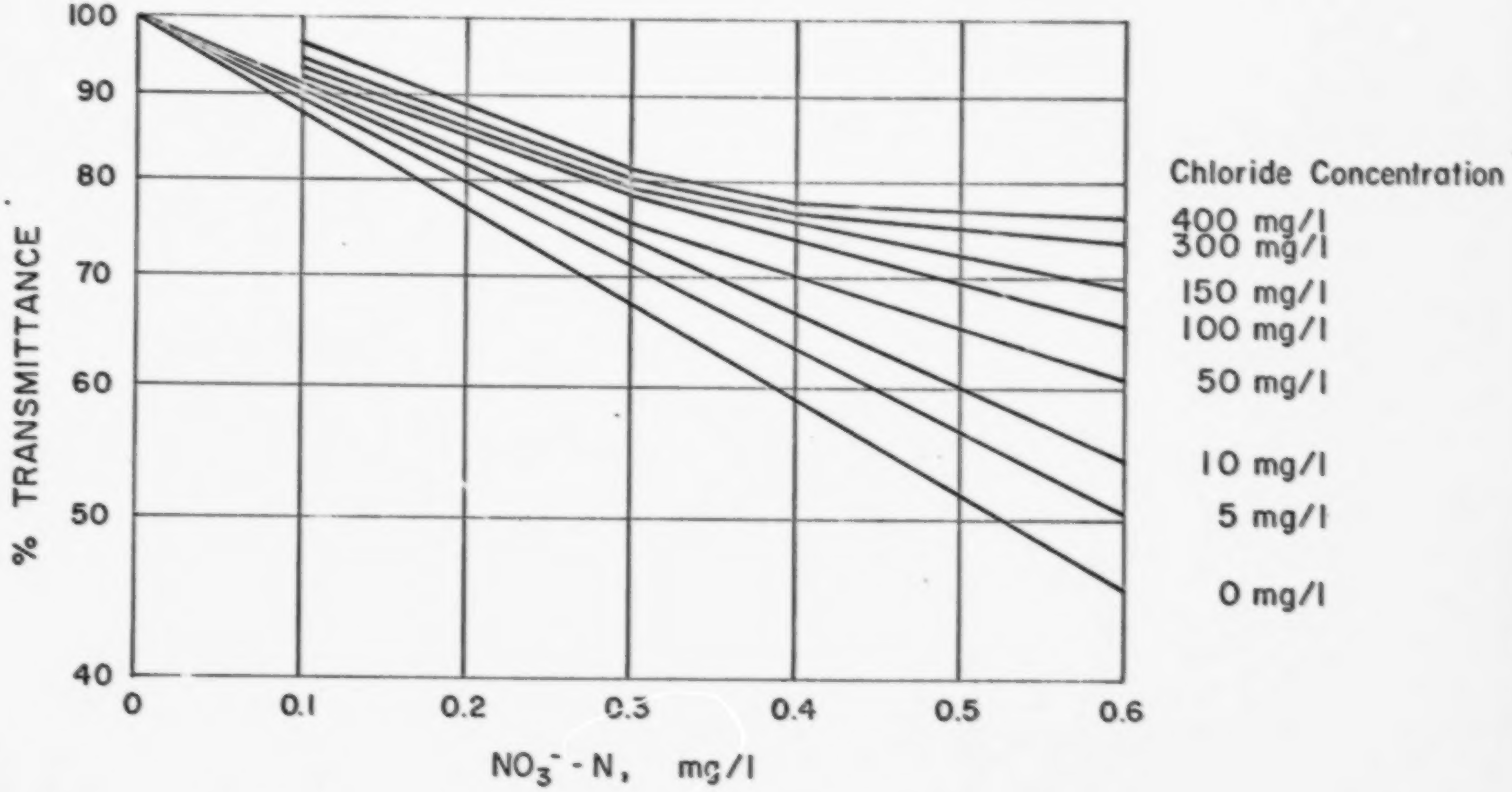

FIGURE 4

Chloride Interference in Phenoldisulfonic Acid NO_3-N Analysis range (with 3 drops of 1.0N NaOH); dissolved the residue in 2.0 ml of phenoldisulfonic acid; heated for 15 min. on a steam bath to dissolve the residue completely; diluted to about 25 ml with distilled water; added 7 ml of 12N KOH and diluted to 100 ml, and the concentrations were determined by reading the transmittance on the spectrophotometer.

The Table in Appendix A presents the observed nitrate concentrations and the percent error at different chloride concentrations in the sample. To use the standard curves, Figure 4, the chloride concentration of the sample was determined to select the standard curve to be used for that sample and the nitrate concentration was read directly from the observed percent of transmittance of the sample.

b. Reduction Method

Some time after this investigation was well underway, a suggestion was made by Lee[26] that consideration might be given to the method of Strickland and Parsons[27] for the determination of nitrate-nitrogen, since with this method the chloride ions do not cause any interference. The method works on the principle that the nitrate ion in the sample is reduced to the nitrite form when the sample is run through a column containing amalgamated cadmium filings. The nitrite formed is then determined colorimetrically after the formation of a highly colored azo dye. A correction can be applied for any nitrite initially present in the incubated sample.

Because of the lack of familiarity with this method some ancillary studies were initiated to check it over and particularly to establish its possible applicability to the investigation at hand. Such items as column design, flow rate through the column and column rinse techniques were found to be critical. However, a standard curve was developed and it was possible to obtain fairly reproducible results consistently. At this time the bulk of the investigation had been completed and it was decided to use the phenoldisulfonic acid test for the remainder of the work for the sake of uniformity. Only a few nitrate determinations were actually made by this method and these are indicated in the data presented further on in this report. However, it was learned that this reduction method has a real potential for nitrate determinations once an analyst has gained a familiarity with it, and it should be given serious consideration particularly when high chloride concentrations may be a problem.

2. Nitrite-Nitrogen

All the nitrite-nitrogen determinations were conducted by the colorimetric diazotization method according to the recommendations of Standard Methods.[17] This test was conducted most frequently of all the nitrogen analyses since it is easily accomplished and is very accurate. All transmittance readings were again made on a Model 14, Coleman Universal Spectrophotometer using matched 13 mm square cuvettes.

3. Total Kjeldahl and Ammonia Nitrogen

Total kjeldahl nitrogens were run directly on the effluent on the days the test runs were set up by a micro-kjeldahl procedure recommended by Perrin.[28] This procedure, which is quite similar to the macro-method recommended in Standard Methods,[17] has the primary advantage that less time is required to obtain results. The magnitude of kjeldahl nitrogen reported is the average of anywhere from four to nine individual analyses depending on the test series. A standard glutamic acid solution was set up at the same time as the effluent sample as a control on the procedure. On the basis of this standard solution and the replicate analyses, it was certain that a reliable kjeldahl nitrogen determination was being obtained.

The kjeldahl nitrogen value includes both the organic nitrogen and the ammonia nitrogen or, in other words, all

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the nitrogen present in the tri-valent negative state. It was of interest to know how much of this form of nitrogen was present in the diluted effluent at the start of each test run since it is this nitrogen form which undergoes the nitrification transformation. With this value known it was then possible to estimate quite closely the final magnitude of nitrate-nitrogen to be expected in the final stages of incubation, that is, when the nitrification stage has been completed. Whether the reduced form of nitrogen at the start of the test run was present in the diluted sample as ammonia nitrogen or organic nitrogen and the relative amounts of each was not of concern, since both forms are eventually oxidized to the nitrate ion. Therefore, only the kjeldahl nitrogen determination was obtained at the time each test series was set up.

Kjeldahl nitrogen analyses were also conducted on the diluted sample at the end of each test run incubation period. These analyses were conducted according to Standard Methods[17] since the very low concentration of kjeldahl nitrogen remaining in these samples made the micro-method very unreliable. It was found that even the macro-method was not sufficiently sensitive to obtain reliable results--judging from the somewhat erratic answers that were recorded. This was not surprising since the combination of very low kjeldahl nitrogen concentrations, variations in the blank correction, and the fact that the final answer must include a five-fold multiplication factor because of the initial dilution, can only result in inherent and unavoidable variations in the final data regardless of the care exercised by the laboratory analyst. Also, it was not possible to run replicate determinations because of the limiting amount of the incubated sample available.

Because of the questionable nature of these kjeldahl determinations it was not possible to obtain a reliable accounting of the various nitrogen forms at the start and end of each of the test runs. At the start of the test run most of the nitrogen can be accounted for in the kjeldahl form since the nitrite and nitrate values were quite low. At the end, however, most of the nitrogen can be accounted for in the nitrate form. Thus, these two determinations were compared to account for the nitrogen, and for all of the test runs, the comparison was felt to be reasonable.

Because of the special nature of the last test series, ammonia nitrogen determinations were also conducted by the micro-method. This method again was selected because of the limited amount of incubated sample available. As in the other test series, the initial kjeldahl nitrogen was run on the effluent directly by the micro-method and the final kjeldahl on the diluted sample by the macro-method.

4. Chloride

All chloride determinations were made by the argentometric method as described in Standard Methods.[17] As mentioned previously, numerous chloride analyses were made to assess the degree of their interference in the nitrate-nitrogen determination.

D. Constant Temperature Incubators

On the basis of the past faborable experience and for the sake of economy, it was decided to construct the constant temperature incubators using commercial refrigerators. This was accomplished by replacing the normal refrigeration temperature controller with one which is considerably more sensitive. In addition, a fan was installed for continuous air circulation. Set up in this manner, constant temperatures in the range of just above 0°C to approximately 30°C can be maintained with reasonable accuracy. The fan motor supplies sufficient heat to adequately maintain the higher temperature. For higher temperatures, a completely covered low wattage (10 to 15 watts) light bult was employed. The wiring diagram employed for the conversion of the commercial refrigerators into constant temperature incubators is presented in Figure 5.

During the test runs the temperatures in the incubators were recorded daily. The thermometers used were calibrated to one-tenth of a degree centigrade and were completely immersed in

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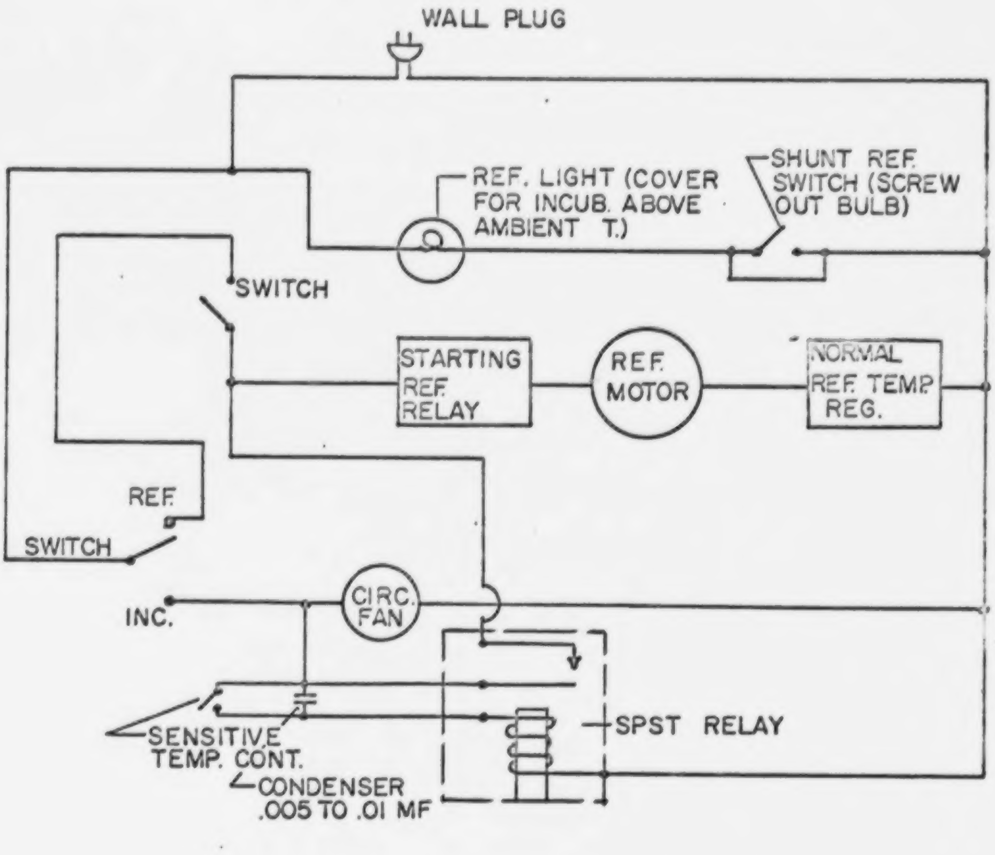
WALL PLUG
REF. LIGHT (COVER FOR INCUB. ABOVE AMBIENT T.)
SHUNT REF. SWITCH (SCREW OUT BULB)
SWITCH
STARTING REF. RELAY
REF. MOTOR
NORMAL REF. TEMP. REG.
REF.
SWITCH
INC.
CIRC. FAN
SPST RELAY
SENSITIVE TEMP. CONT.
CONDENSER .005 TO .01 MF

Wiring Diagram for Constant Temperature Incubator

FIGURE 5

water filled tubes. A summary of the temperature data for the entire investigation is presented in Table 5. As noted the table shows the duration of each test run in days, the average of the daily recorded temperatures and the maximum range in recorded temperatures. On the whole it was felt that the temperature control was very satisfactory. The average recorded temperature in many cases was equal to the nominal test run temperature and in the remaining cases the maximum disparity amounted to only 0.2°C. In the majority of the cases the maximum range in temperature was about 1°C and in no case was it greater than 1.7°C. Also these extremes occurred only once or twice during each test run and usually during periods when the incubators had to be opened repeatedly to obtain samples. Eliminating these extremes would lower appreciably the extent of temperature variations. Because of this effective temperature control, it will be assumed that the actual test run temperature in all cases is exactly equal to the nominal temperature throughout the remainder of this report.

E. Setting Up Test Series

Preparation for the setting up of a test series usually commensed some two weeks in advance of the actual set-up date. The incubators were first adjusted to the test run temperature. Secondary effluent from the Milwaukee plant was acclimated to the test run temperature. Distilled water in five gallon

TABLE 5

Incubation Temperature Data for Duration of Each Test Run

Test Series	Test Run	Duration of Test Days	Avg. Temp °C	Temp. Range°C (Maximum)
A	II-10	91	10.2	9.6-10.6
	II-20	49	20.0	19.6-20.2
	II-30	49	29.9	29.9-30.2
B	IV-5	154	5.0	4.3-6.0
	IV-20	41	20.2	19.3-20.9
	IV-35	39	34.9	34.3-35.5
C	VI-15	37	15.0	14.4-15.6
	VI-20	37	20.0	19.8-20.3
	VI-25	37	25.0	24.5-25.5
D	VII-10	87	10.0	9.4-10.7
	VII-20	66	20.1	19.5-20.6
	VII-30	38	30.0	29.4-30.5
E	XI-10	91	10.0	9.6-10.5
	XI-15	91	15.0	14.5-15.4
	XI-20	56	20.1	19.4-20.5
	XI-25	49	24.9	24.0-25.6
	XI-30	49	30.0	29.1-30.4
F	I-10	65	10.1	9.6-10.6
	I-15	65	15.0	14.6-15.5
	I-20	54	20.1	19.2-20.5
	I-25	50	25.2	24.2-25.8
	I-30	50	30.1	28.4-30.6
G	III-10	68	10.0	9.5-10.6
	III-15	68	15.1	14.6-15.6
	III-20	45	20.1	19.7-20.5
	III-25	45	25.1	24.2-25.8
	III-30	45	30.2	29.4-30.6
H	V-10	38	10.1	9.6-10.6
	V-20	30	20.0	19.7-20.5
	V-30	30	30.1	29.0-30.6
J	VI-10	42	10.1	9.6-10.6
	VI-20	42	20.2	19.9-20.5
	VI-30	42	30.1	29.1-30.6

carbovs was also placed into the incubators and after reaching the incubator temperature, the water was aerated to saturation. All the glassware which was used for incubating diluted sample, that is the carbovs and the BOD bottles, were washed with chromic acid solution and thoroughly rinsed first in an inverted position with hot tap water followed by several rinses with distilled water.

On the day of the actual test series set-up, the grab effluent sample was brought into the laboratory and the temperature and the pH were immediately measured. The general visual characteristics of the effluent were also noted. The effluent was then carefully divided into containers representing the number of test runs to be set up in the particular series. In all the series except E, F and G it happened to be three containers; whereas for the series mentioned it was five. The effluent was carefully apportioned into the separate containers to insure that the effluent characteristics were as identical as possible for each of the test runs. This was accomplished by filling each test run container in small increments from each of the effluent jugs brought into the laboratory while stirring the jug contents vigorously at all times during the operation. Sufficient sample was then obtained from these containers for conducting the chloride, kjeldahl-nitrogen and any other initial analyses which were run on the effluent directly. A summary of the pertinent data on the effluent samples employed for the entire investigation plus other general test run information is presented in Table 6 and the previously presented Table 5.

The effluent sample was acclimated to the particular test run temperature and aerated to saturation. After adding the necessary salts to the distilled water, the proper measured volume of effluent and dilution water were added to the 5 gallon carboys to make up the final 20 percent dilution. Finally, the concentrated acclimated seed was added to the mixture. A magnetic stirring rod was retained permanently in the carboy. After mixing and aerating the final dilution, sample was obtained for the initial chemical analyses and approximately 15 BOD bottles were filled. The initial DO was determined using two of the bottles, and the remaining bottles were incubated with the jug after filling the bottle wells with water and sealing the tops tightly with plastic food wrap and rubber bands.

Because of the different incubation temperatures and the varying effluent characteristics from series to series, no attempt was made to use a similar time sequence for running the BOD and nitrogen analysis in all of the test runs. Rather, an attempt was made to follow deoxygenation progression as closely as it was practically possible. To conserve sample, usually only one BOD bottle was used for the DO measurement on the day it was decided to run this particular analysis. A second or in some

TABLE 6

SUMMARY OF PERTINENT INITIAL DATA ON MILWAUKEE SEWERAGE COMMISSION SECONDARY EFFLUENT USED DURING RESEARCH PROJECT

Test Series	Day & Time Grab Sample Obtained	Temp °C	pH	Chloride Conc. mg/1	Total Kjeldahl Nitrogen mg/1	5-Day 20°C BOD mg/1	General Remarks
A	2/26/66 Sat. 10:00 a.m.	13	7.4	189	7.37	11	Yellow Color--Flocky matter in suspension
B	4/23/66 Sat. 9:30 a.m.	17	7.2	157	5.66	20	Some flocky matter in suspension--Slight rain during early morning
C	6/7/66 Tues. 10:00 a.m.	22	6.6	132	7.93	23	Light yellow tint--Some flocky solids in suspension
D	7/20/66 Wed. 10:00 a.m.	23	7.1	122	4.25	19	Light yellow tint--Very little matter in suspension
E	11/12/66 Sat. 10:00 a.m.	16	7.4	127	7.57	11	Faint yellow color--Very little particulate matter in suspension
F	1/26/67 Thur. 9:30 a.m.	13	7.3	162	6.8	14	Yellow color--Slightly turbid--Few large flocky solids
G	3/22/67 Wed. 9:30 a.m.	14	--	204	5.53	13	Relatively clear Some turbid matter and flocky solids--Probably contains some melted snow and ice

41-b

TABLE 6

SUMMARY OF PERTINENT INITIAL DATA ON MILWAUKEE SEWERAGE COMMISSION SECONDARY EFFLUENT USED DURING RESEARCH PROJECT
(continued)

Test Series	Day & Time Grab Sample Obtained	Temp °C	pH	Chloride Conc. mg/1	Total Kjeldahl Nitrogen mg/1	5-Day 20°C BOD mg/1	General Remarks
H	5/13/67 Sat. 10:00 a.m.	18	7.4	140	4.37	10	Light yellow color--Turbid but not containing large flocky solids
J	6/21/67 Wed. 9:30 a.m.	22	7.1	125	2.83	5	Light yellow tint--Little flocky matter in suspension

cases even a third bottle was employed when the results appeared erratic or inconsistent with the previous analysis. One advantage of the "jug" dilution procedure is that the deoxygenation progression can be monitored satisfactorily with less duplication of analyses, since the same diluted mixture is being used throughout the test run. At higher incubation temperatures shorter incubation intervals were used between analyses because of the higher rate of deoxygenation progression. The slower rate at lower temperatures meant that longer incubation periods had to be used to conserve diluted sample for the longer duration of these test runs. Both DO and nitrogen analyses were made at greater frequency at the transition between the carbonaceous and nitrogenous stages in an effort to delineate this activity as accurately as possible. Usually a larger number of nitrite-nitrogen analyses were conducted in the earlier stages of deoxygenation, whereas the nitrate-nitrogen analyses were more numerous at the later stages. Actually it required the experiences gained from some of the earlier test series before a testing program was set up that was considered to be as close to the optimum as possible.

When the dissolved oxygen in the sealed bottles reached about 2 mg/1, the contents from the remaining bottles were poured into the jug and the mixture was once again reaerated to saturation. At this time a minimum of two and usually more DO analyses were conducted prior to, and following reaeration, because of the importance of these magnitudes in the final calculations of the BOD. A new batch of sealed BOD bottles were again incubated along with the large jug for the continuous monitoring of the deoxygenation progression.

F. Final Testing Program

At the outset of the study it was planned to set up test runs at temperatures ranging from 2° to 45°C and to set up three different temperatures in each test series. In order to more effectively compare the results of different test series, it was decided to always include a 20°C test run in each of the series. In this way the data of each series could be represented relative to the 20°C data, and thus facilitate comparison of results between series. Thus series A, B, C and D were set up in this fashion and included temperatures from 5° to 35°C, at 5° increments. Upon a preliminary review of the results of these four series, it was decided to run a shorter incubation range; namely, 10° to 30°C, and to set up more test runs in each series. Thus, the added series E, F and G, each included the incubation temperatures 10°, 15°, 20°, 25° and 30°C. These changes were made for the following reasons. First, the temperatures below 10°C required an extremely long test run duration to "complete" the deoxygenation progression. The rate of secondary effluent deoxygenation at such temperatures is so slow that the use of such information, in a practical situation, would be rather limited. Practical applications above 30°C were also felt to be limited. Though the study of these temperature extremes would be of academic interest, it was felt that more attention and effort should be directed to the more practically significant temperature range of 10° to 30°C.

Second, it was decided that using more temperatures in each series and repeating the series several times would more accurately establish the constants of the deoxygenation kinetics. In spite of the fact that a 20°C test run was included in the first four series, differing effluent characteristics made it difficult to analyze the different series to make up the full range of temperatures. By including the complete temperature range in one series (as in test series E, F and G) it was possible to analyze each as a single unit in addition to comparing one with the other.

The last two series, that is, series H and J were set up to investigate principally the mechanism of the nitrification phase of deoxygenation. They both included incubation temperatures of 10, 20 and 30°C and involved only nitrogen analyses. BOD determinations were not included in these two series because of the limited incubator space and sample abailable. Series H involved the setting up of four jug dilutions in the conventional manner with four different seed quantities as follows: no seed, 10, 20 and 30 ml of seed per liter of diluted sample. The seed was added in the concentrated form as discussed previously, thus the above values are actually equivalent quantities. The progress of nitrite and nitrate-nitrogen formation was then closely monitored for the different seed quantities at the three incubation temperatures. The primary purpose of this test series was to determine what influence the seed quantity might have on the nitrification phase of the deoxygenation progression, and to possibly apply this information in the final interpretation of the evaluated deoxygenation constants.

In the J series four jug dilutions were also set up in the usual manner but in two of the jugs 5 mg/l of ammonia-nitrogen was added in addition to the varying seed quantities. The four jugs were set up at each temperature as follows:

Jug Dilution	SEED - ml per liter of diluted sample	NH_4Cl added as N (mg/l) diluted sample
1	5	0
2	25	0
3	5	5
4	25	5

Again the seed was added in the concentrated form so that the amounts shown are actually the equivalent quantities. In addition to following the nitrite and nitrate-nitrogen progression, ammonia-nitrogen analyses were also conducted in this test series. The purpose of this particular series was to assess the effect of not only the seed quantity but also of the available ammonia-nitrogen on the nitrification activity.

V. TEST RESULTS

All the final deoxygenation test results are included in 33 different test runs which are part of 9 different test series. The laboratory analyses are summarized in Tables B-1 through B-36 which are presented in Appendix B of this report. An indication of the magnitude of this investigation can be gained from the fact that the analyses include approximately 700 BOD determinations, 1250 nitrite-nitrogen determinations, 500 nitrate-nitrogen determinations and 70 ammonia nitrogen determinations. These numbers do not include the large number of determinations made for the ancillary studies and the fact that duplicate or greater replicate determinations were required on many occasions.

Though all of the analyses were conducted on the diluted effluent taken directly from the "Jug," the results are expressed in terms of the effluent concentration in milligrams per liter (mg/l). Thus the concentrations were figured taking into account the 20 percent dilution in all cases. Series A through G include BOD, nitrite-nitrogen and nitrate-nitrogen determinations for the various incubation periods, whereas, series H and J include only the nitrogen analyses.

All the reported test runs are identified using three difficult designations. For example, the heading "Test Run A-II-20" means that the particular test run is part of Series "A" which was set up in the second (II) month of the year, or February, and the nominal incubation temperature was 20°C.

VI. ANALYSIS OF RESULTS

Having the BOD and nitrogen data available at the various incubation temperatures, the next step was to calculate the monomolecular curves which best fit the observed data. It was noted at the outset of this report, that because of its traditional usage and general acceptance, it will be assumed that both stages of deoxygenation proceed according to a monomolecular or first order reaction. Some method had to be selected of the numerous ones reported in the literature for the evaluation of the deoxygenation constants, k and L. The deoxygenation constants for both the carbonaceous and nitrogenous stages could only be evaluated after a decision was made as to the procedure to be employed for the separation of the two stages using the BOD and nitrogen data together. A means of establishing the time of nitrification onset had to be also decided, and applied with a consistency to all of the test run data. These procedures together with a detailed presentation of the results of the individual test runs, plus a summary of all the evaluated constants, are presented in the following sections of this report.

A. Evaluation of Deoxygenation Constants (k) and (L)

Numerous methods for the evaluation of the monomolecular velocity constant, k, and the ultimate demand, L, have been reported in the literature through the years. However, the most accurate and generally accepted methods are those developed by Reed and Theriault,[2] Thomas,[29] and Moore, Thomas and Snow.[30]

The last two methods are generally referred to as the "slope" method and the "moments" method respectively. The first two methods employ the well-known statistical technique of the "least squares" to establish the line of best fit; whereas, the latter method employs a less tedious technique, that being the taking of moments of the observed BOD values about the vertical coordinate axis of the BOD vs. time curve. Studies by Schroepfer et al[12] concur, that from the standpoint of both practicality and accuracy, the "moments" method is the best of the techniques available. Therefore, all of the deoxygenation constants presented in this report have been evaluated by this particular method.

One disadvantage of the "moments" method is that certain graphs are required to expedite the evaluation of k and L otherwise the procedure becomes one of the trial and error type. These graphs, however, are only applicable for one specified incubation sequence and no other. This is no problem when common incubation sequences as 1, 3 and 5 days, or 1, 3, 5 and 7 days, or 1 through 5 days, etc. are being employed since these graphs are available in many sources. However, because of the different incubation temperatures and the long duration of the tests, by necessity it was not possible to use the same incubation for all of the test runs. This would mean that a large number of graphs would have to be drawn requiring a large amount of tedious computations if the moments method were to be used as originally developed. Instead, for the computations of this investigation, mathematical tables were set up with the aid of a computer which covered all the possible combinations of incubation sequence and velocity constants. These tables were used instead of the graphs which saved a great deal of time. In effect, the computational procedure turned out to be partially trial and error, whereby the mathematical tables were employed to plot a short segment of the necessary graphs through the magnitude range of the velocity constant involved. With a small amount of computational experience the procedure seemed to work quite well and gave results which were consistent and reliable.

In all of the test runs reported the assumption was made that no significant lag occurred in the initial progress of deoxygenation. This is an important assumption since it often happens that the BOD progression with time will exhibit a pronounced lag at the onset of deoxygenation. There are several possible causes for such a lag; the most likely one however is poor or inadequate seeding conditions. In all of the test runs of this investigation, special care was exercised to use a sufficient quantity of well acclimated seed to prevent the occurrence of any perceptible lag. A review of the actual test results will attest the fact that this attention was well warranted since in no case did a significant lag occur, even at the lower incubation temperatures. This fact simplified the procedure for the evaluation of the deoxygenation constants since a modification in the "moments" method would have had to be made if a significant lag had occurred.

B. Separation of Carbonaceous and Nitrogenous Phases of Deoxygenation

One of the important aspects of this investigation was to delineate as accurately as possible the two distinct phases of the effluent deoxygenation at the various incubation temperatures. This is particularly true of secondary effluents since a very large percentage of the carbonaceous matter is removed by the treatment process and often-times the nitrogenous demand is the largest of the two. The nitrogen analyses were used for the purpose of separating mathematically the two stages.

The assumption was made that the oxygen demand exerted during the nitrification phases proceeded quantitatively according to equations (4) and (5) presented earlier in this report. Therefore, according to the first equation, 1-1/2 moles of oxygen must participate in the formation of one mole of nitrite-nitrogen which in terms of BOD means that the nitrite-nitrogen concentration multiplied by 3.43 is equal to the total BOD involved in the oxidation of ammonia-nitrogen to nitrite-nitrogen. The second equation representing nitrate formation shows that two moles of oxygen are required, which makes the conversion factor for BOD equal to 4.57. In the analysis of each of the test runs, the first step was to convert the nitrogen values to equivalent BOD and subtract these demands from the total BOD as determined from the dissolved oxygen analyses of the "jug" dilution technique. The remaining points should represent the progression for the carbonaceous demand. A curve of best fit was then established for these calculated points by the "moments" method, and the carbonaceous velocity constant, k, and the ultimate demand, L, were determined. The theoretical curve was calculated using the established deoxygenation constants, and the calculated BOD was determined at all the incubation periods for which the observed BOD values were available. Subtracting the calculated points from the observed points resulted in a deoxygenation progression assumed to be solely of the nitrogenous phase. The curve of best fit was established for these remaining points to arrive at the nitrogenous velocity constant and ultimate demand.

In the process of establishing the nitrogenous velocity constants, some judgment had to be exercised in the selection of the exact time of nitrification onset. The accurate establishment of this value was critical as the magnitude of this value has a significant influence on the value of the nitrogenous velocity constant. The decision was made to select a time value which according to the judgment, fit in well with the main trajectory of the nitrogenous progression. This meant that in all cases a short transitional phase of nitrification was ignored. The term "active" nitrification was used to signify the portion of the nitrification progression which was actually included in the mathematics of the evaluation of deoxygenation constants. The so called "active" nitrification represented the most significant portion of the entire nitrification phase which is the reason why emphasis was placed on this portion in the evaluation procedure. Once the constants were established, the theoretical curve was calculated and plotted with the observed points. In some cases a new onset time, t_N, was selected and a re-evaluation of the deoxygenation constants was made following an examination of this plot. This procedure was continued until constants were evaluated which seemed to best describe the observed points. In most cases the first evaluation was sufficient.

The fact that a transitional phase exists between the carbonaceous and the nitrogenous activity is well-known. It would be unreasonable to expect an abrupt change from one stage to the other particularly since the biological activity is involved. To attempt to mathematically describe with accuracy this transitional phase would be extremely difficult, and if possible, the resulting equation would be too complicated to be of any practical use. It appears that the best solution is to recognize that this transition does occur, and to use two distinct curves to describe the major portions of the entire deoxygenation activity. This has been the traditional manner in which this has been done in the past--at least when monomolecular or first-order reaction rates are used to describe the reaction kinetics.

In some studies of the past, attempts were made to separate the two stages of deoxygenation without the availability of the associated nitrogen analyses. This can be accomplished in an approximate manner by the judicious selection of observed BOD values in the evaluation procedure. A great deal of judgment had to be exercised as to the possible trajectory of the deoxygenation kinetics of the two stages. From the experiences of this investigation, it can be stated that an attempt to separate the two stages, without associated nitrogen analyses, would be very difficult, and at best, only a rough approximation of what is actually happening. Some test runs experienced more pronounced transitional phases than others. In test runs at the same temperature nitrification activity commenced at varying times, and in one test series, both stages appeared to progress simultaneously at the time the test runs were set up. The nitrification activity varied markedly with temperature so that the same pattern could not necessarily be expected for all of the test runs of the same series. All of these factors point out the hopelessness of achieving a reasonably accurate interpretation of deoxygenation data without the availability of the associated nitrogen analyses--particularly in the situation where the substrate is a secondary effluent.

In some instances after subtracting the nitrogenous from the total demand, the resulting points were somewhat out of phase with the general trend of the carbonaceous projection.

This problem usually occurred at the transition point where high nitrite values began to affect the accuracy of the nitrate determinations. In such cases where the analyses might be considered questionable, the observations were not included in the evaluation procedure. This situation did not occur frequently. Usually, in the early stage of nitrification, nitrite formation is significant and the nitrate formation is nominal. At the later stages of deoxygenation, however, the reverse is true.

C. Results of Individual Test Series

Before discussing the results of each individual test series, a few explanatory comments can be made which would be applicable to all of the results. In the deoxygenation plots to be presented, all of the observed BOD values are shown. The calculated curves presented, and the deoxygenation constants for both stages, were evaluated by the procedure already described. The point of nitrification onset selected is also presented for each of the test runs. Though in some cases a fractional number of days could have been selected for this value, it was decided that such a refinement would be unwarranted in view of the arbitrary manner in which the value was selected. Therefore, this value was held to a whole number of days. The carbonaceous curve extended beyond the junction of the two stages is shown as a dashed line, since this is merely a calculated extension of this stage and represents only a portion of the total activity in this range.

1. Test Series A-II

The results of the test series A-II is shown in Figures 6, 7, and 8. This was the first series set up and in a sense was somewhat of an exploratory nature which accounts for the relatively few observations in the carbonaceous portion. It should be remembered, however, that in the evaluation of the deoxygenation constants a much larger number of observations are added since the points representing the total observations minus the nitrogenous demand would be included.

2. Test Series B-IV

The results of test series B-IV are shown in Figures 9, 10 and 11. This test series included incubation temperatures of 5° and 35°C, which are the only runs of the entire investigation conducted at these temperatures. The duration of the 5°C test run was the longest of all of the test runs conducted.

3. Test Series C-VI

The results of test series C-VI are presented in Figures 12, 13, and 14. From an examination of the calculated carbonaceous curves in the three plots, it would appear that they do not accurately describe the deoxygenation progression. However, in this test series, a small degree of nitrification started also immediately and continued at a steady pace throughout the intial phase of the deoxygenation.

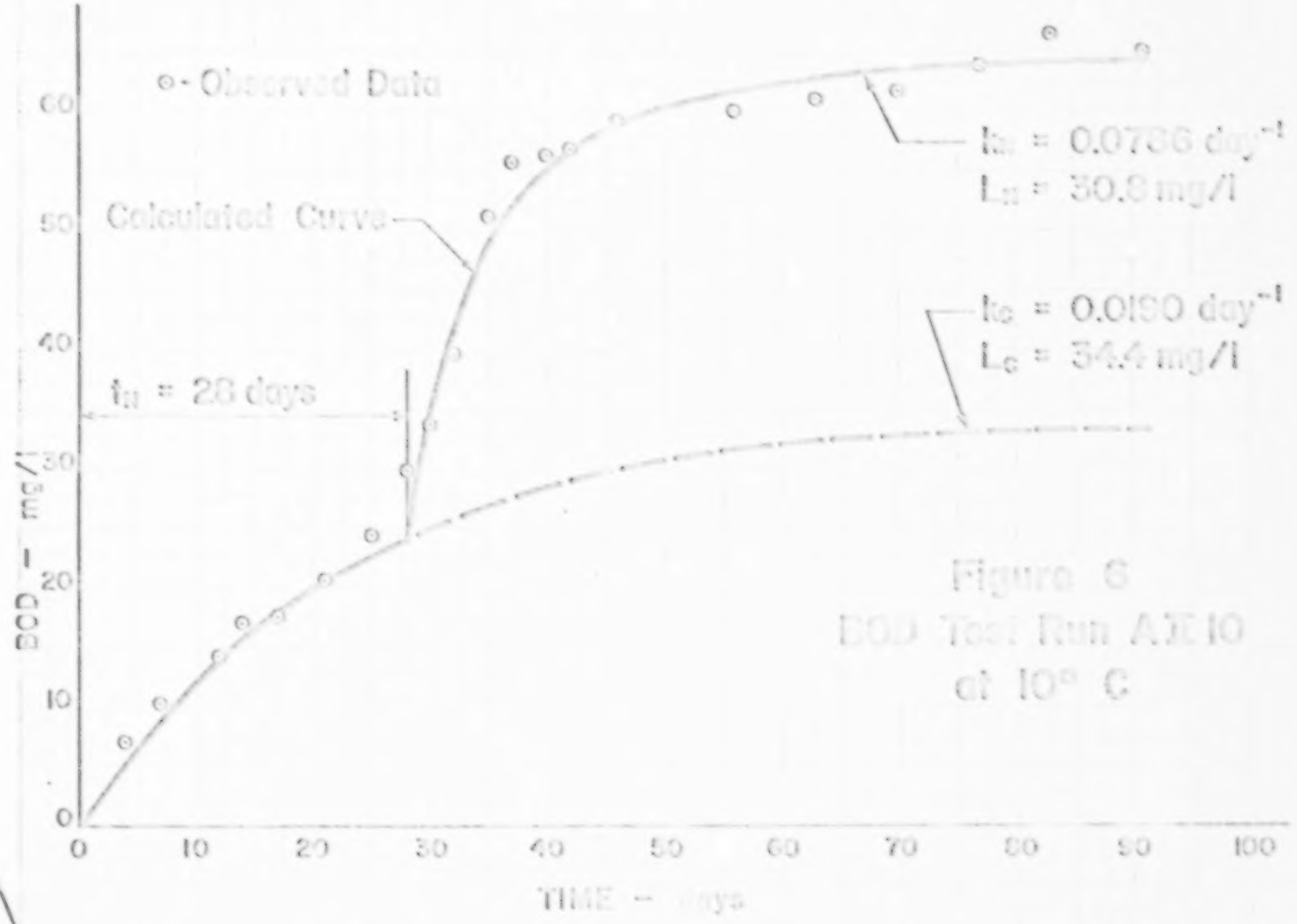

Figure 6
BOD Test Run A X 10
at 10° C

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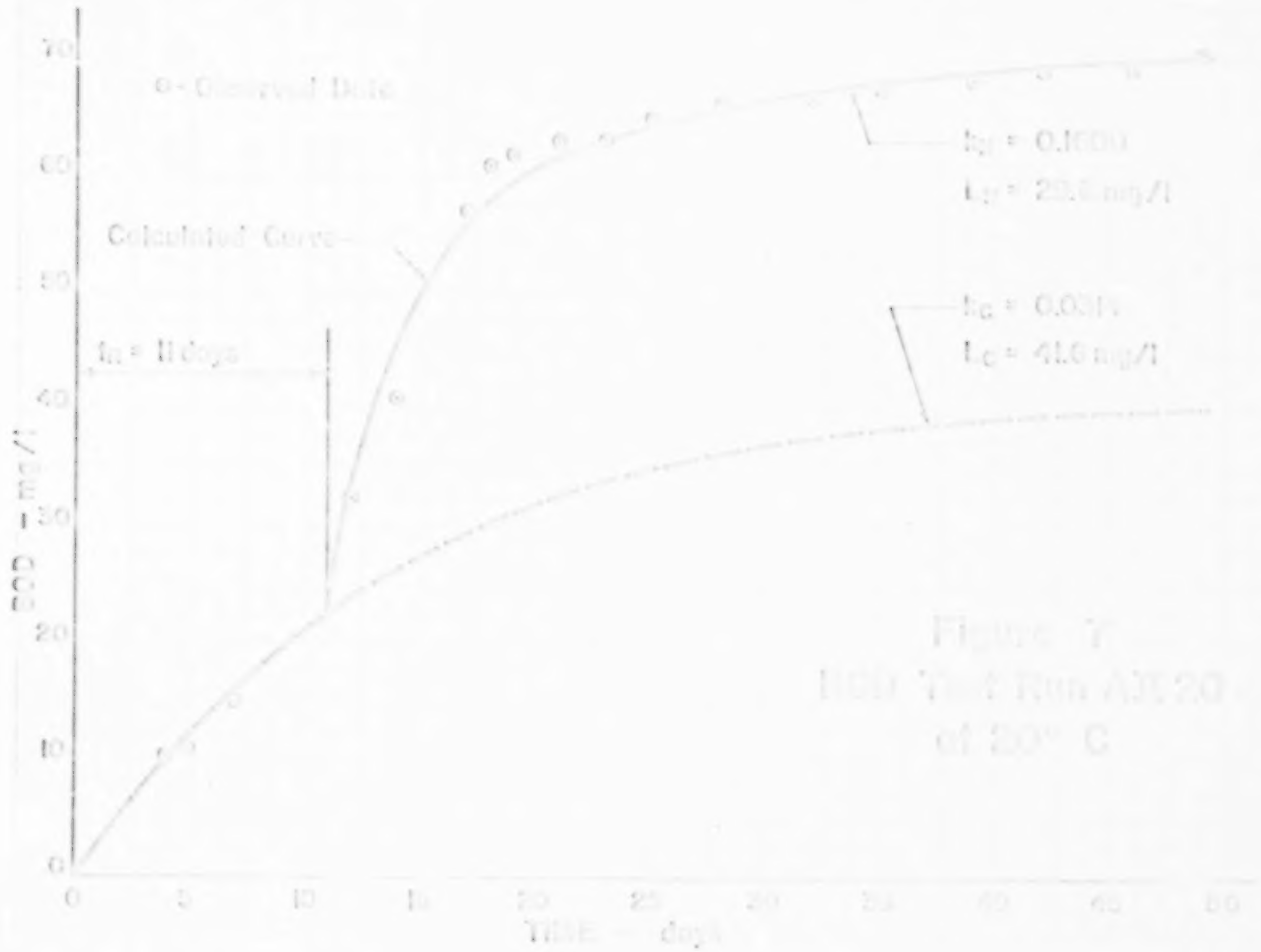

Figure 17
BOD Test Run AJ.20
at 20° C

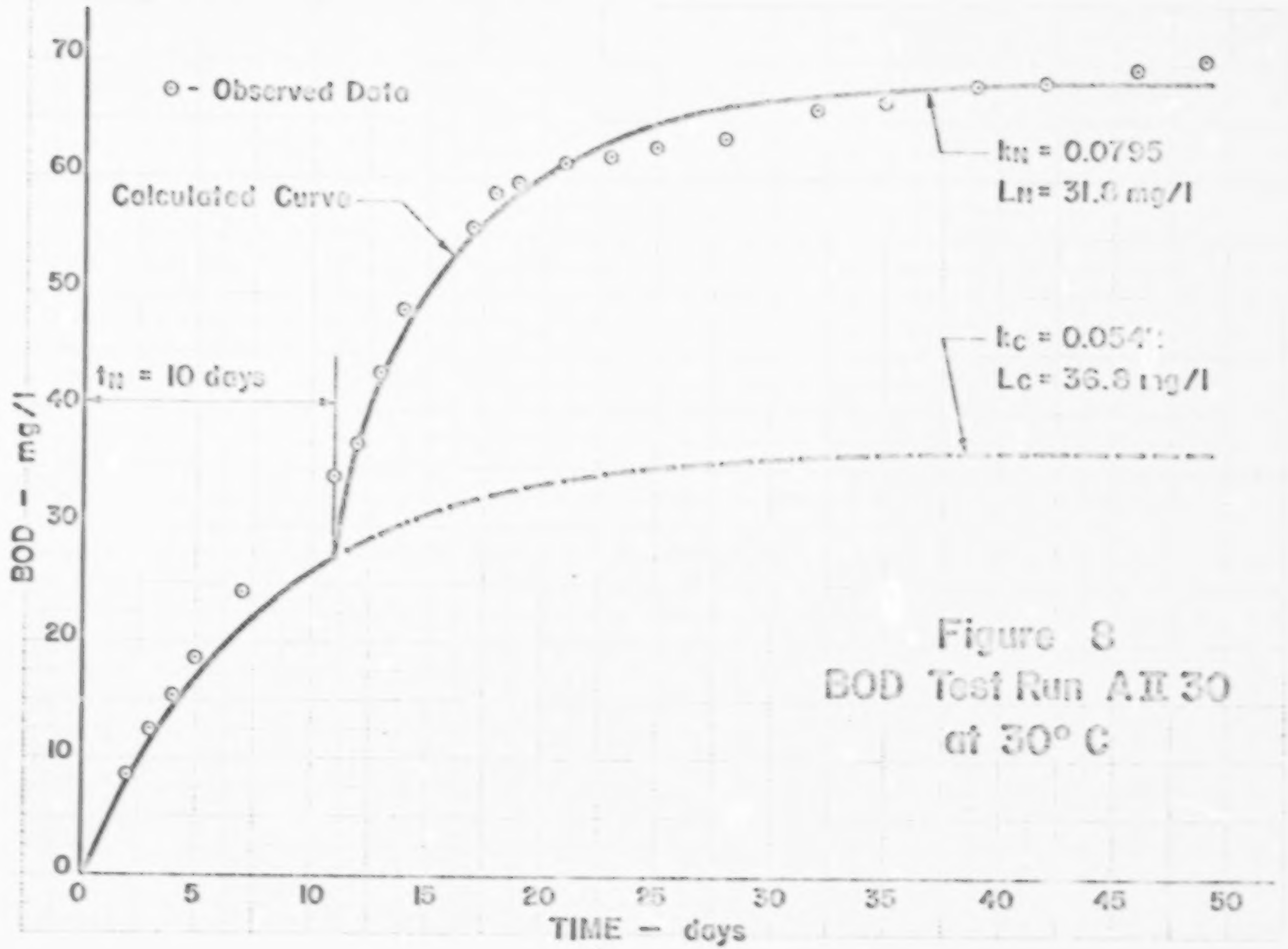

Figure 8
BOD Test Run A II 30
at 30° C

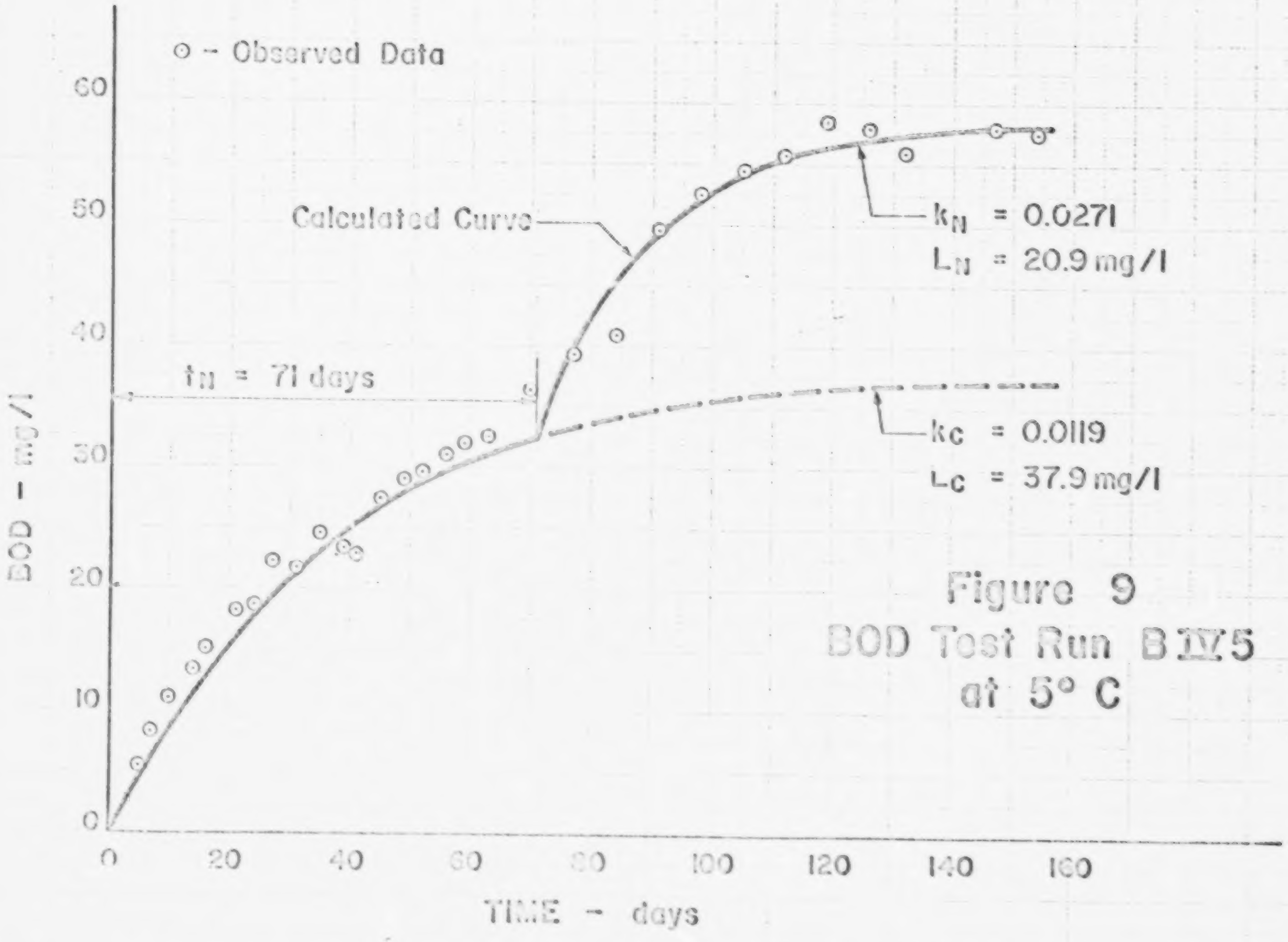

Figure 9
BOD Test Run B IV 5
at 5° C

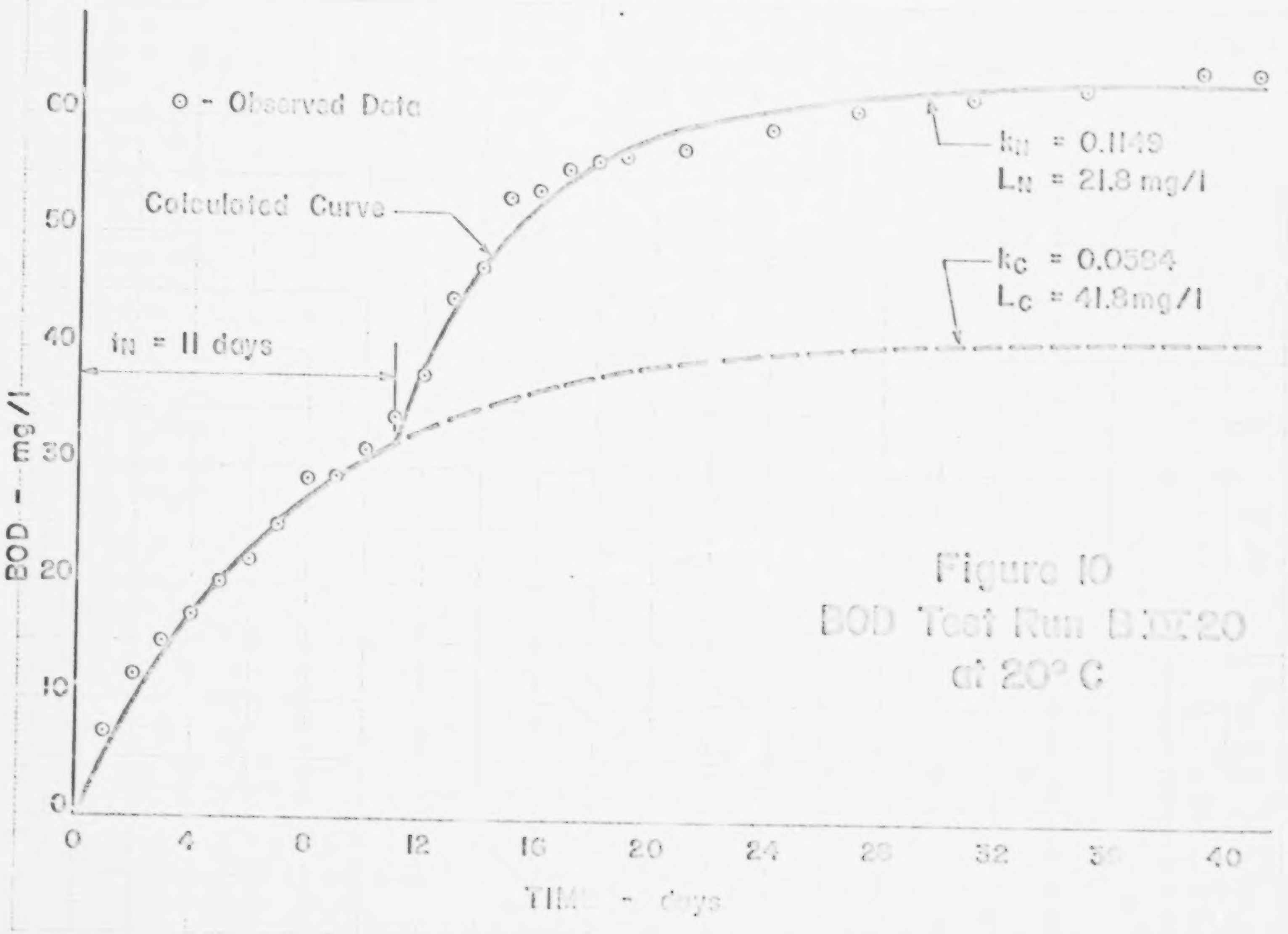

Figure 10
BOD Test Run B IV 20
at 20° C

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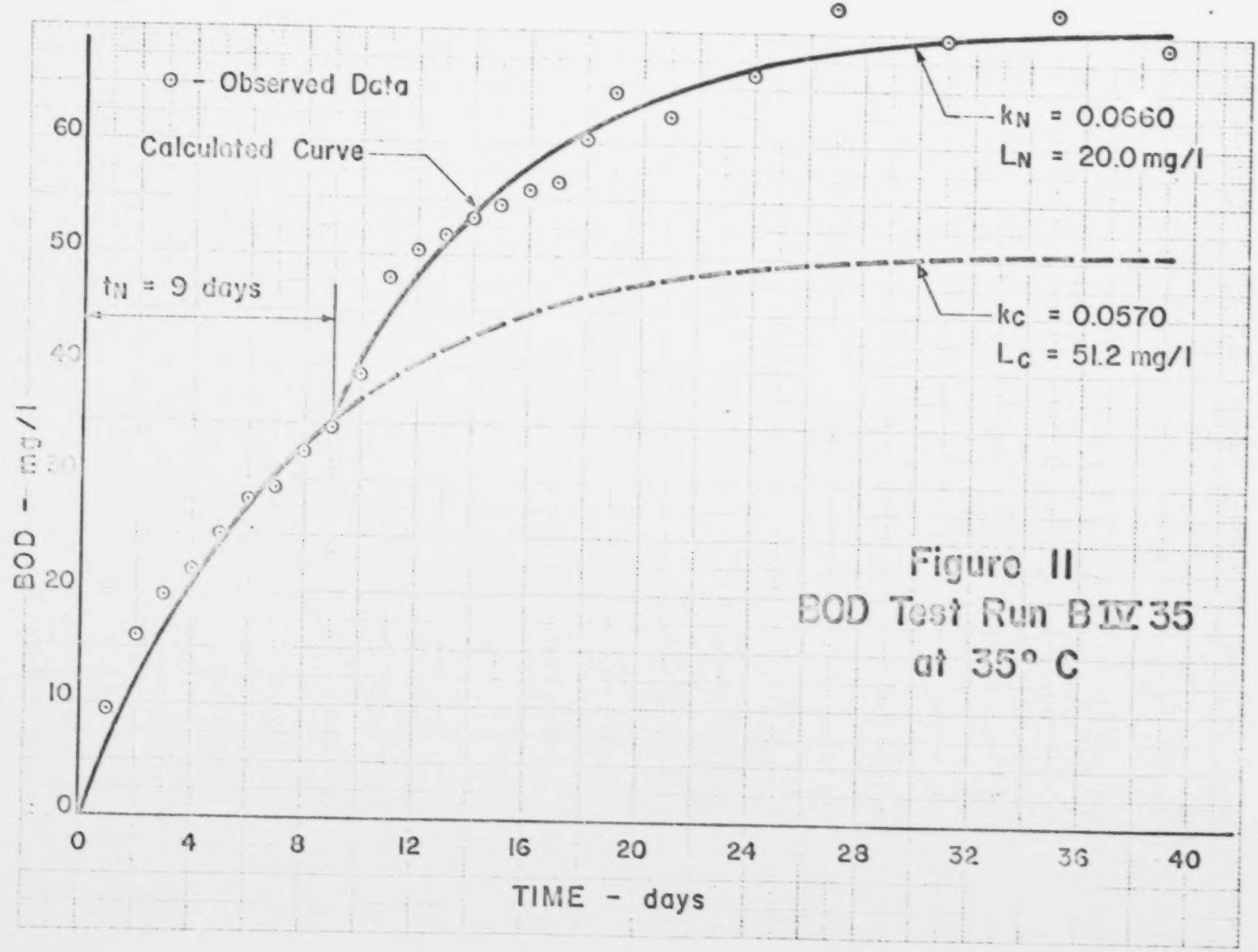

Figure 11
BOD Test Run B IV 35
at 35° C

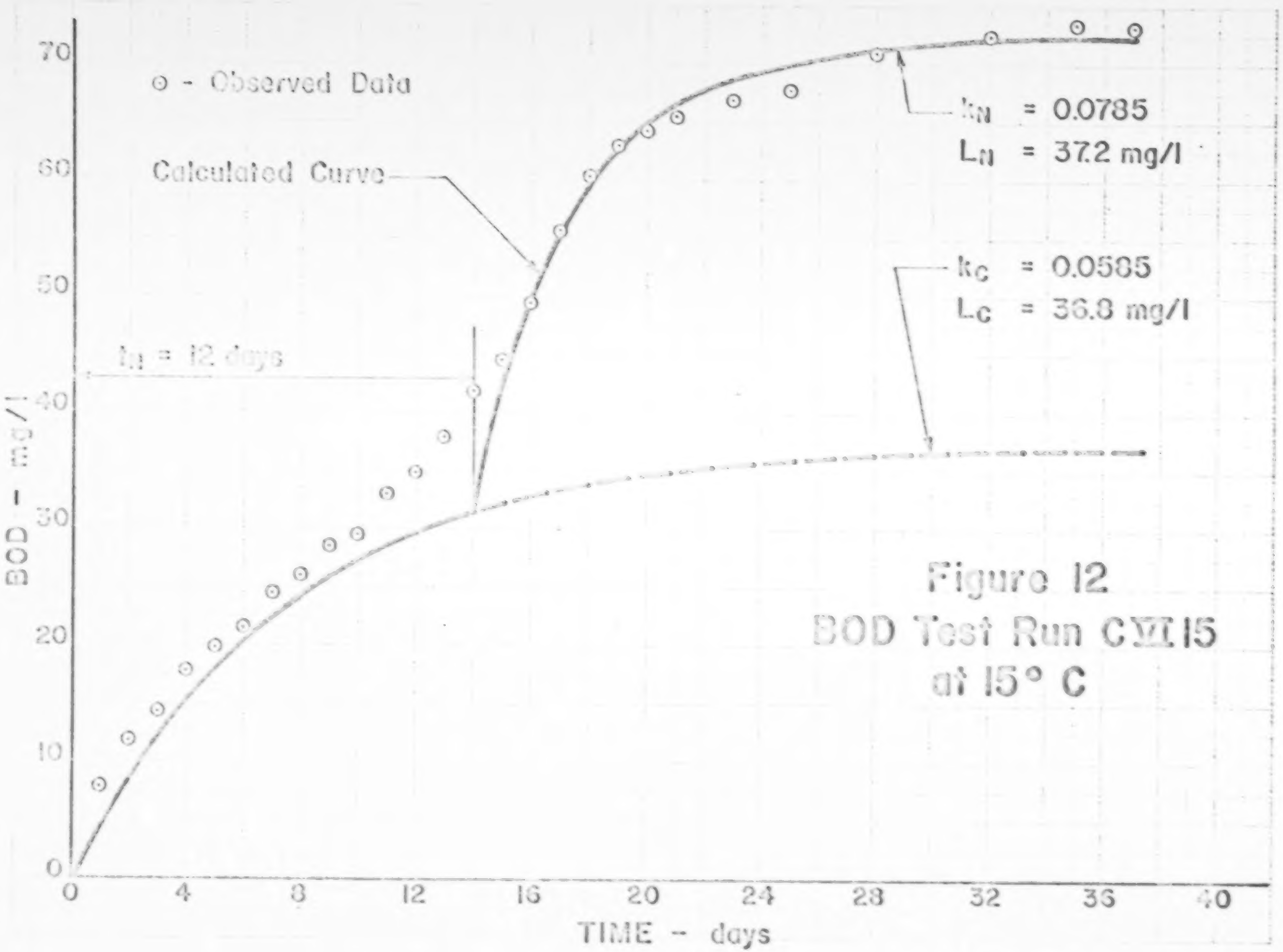

Figure 12
BOD Test Run CVII 15
at 15° C

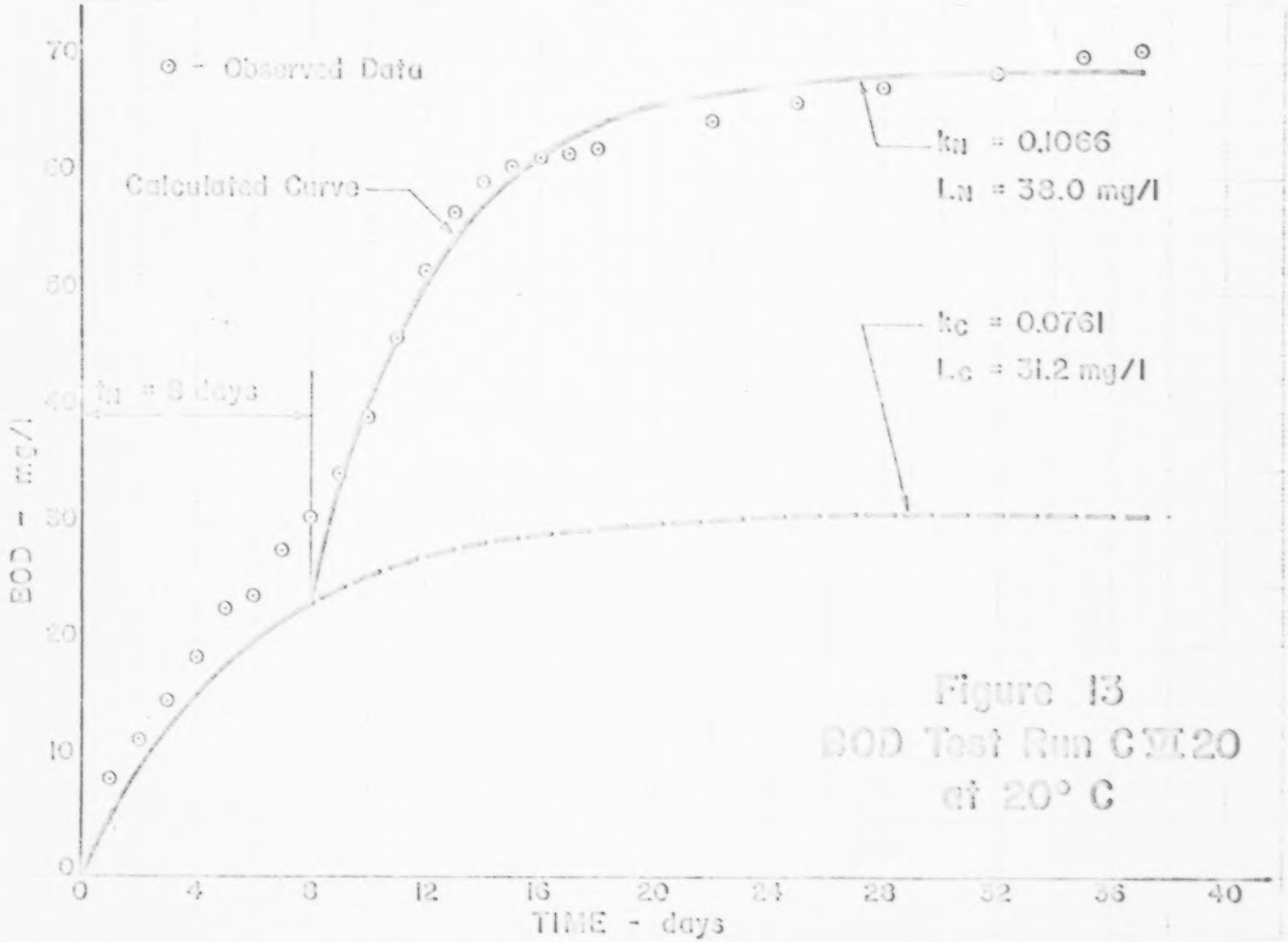

Figure 13
BOD Test Run C VI.20
at 20° C

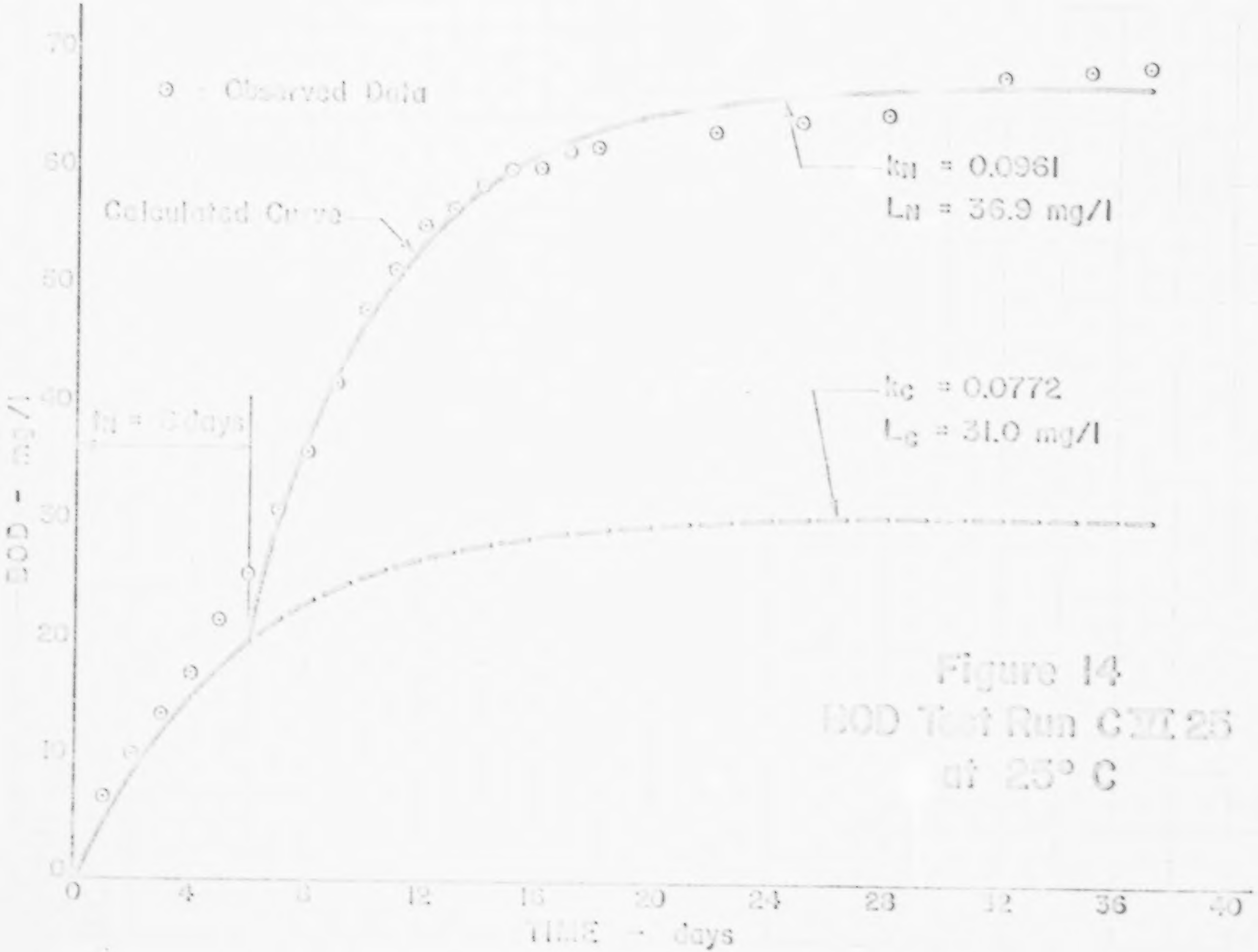

Figure 14
BOD Test Run C XII 25
at 25° C

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Since the nitrogenous demand was subtracted from the total demand in all cases, the actual carbonaceous activity would be below the observed points. Also, the calculated carbonaceous points within the active nitrification stage tended to force the calculated curve downward--a fact which is not actually apparent in the figure. These curves demonstrate the distinction between the lower level nitrification activity, which can occur from the onset of the test run right on through the transitional phase, and the so-called "active" phase which was given primary attention in this investigation. The pattern evident in this test series also occurred in others, but to varying degrees. In spite of these generalizations, the calculated curves still appear to describe the entire deoxygenation progression with reasonable accuracy.

4. Test Series D-VII

The results of the D-VII test series appear to be the most unusual of all the test series. These results are presented in Figures 15, 16, and 17. As noted, at all the three incubation temperatures, the time to nitrification onset was extremely short. In the case of 30°C, the value was taken as zero days, indicating that both stages proceeded at their maximum rate from the start of the test run. The reason for this behavior is not known. The seeding practice was about the same as for the other test

15

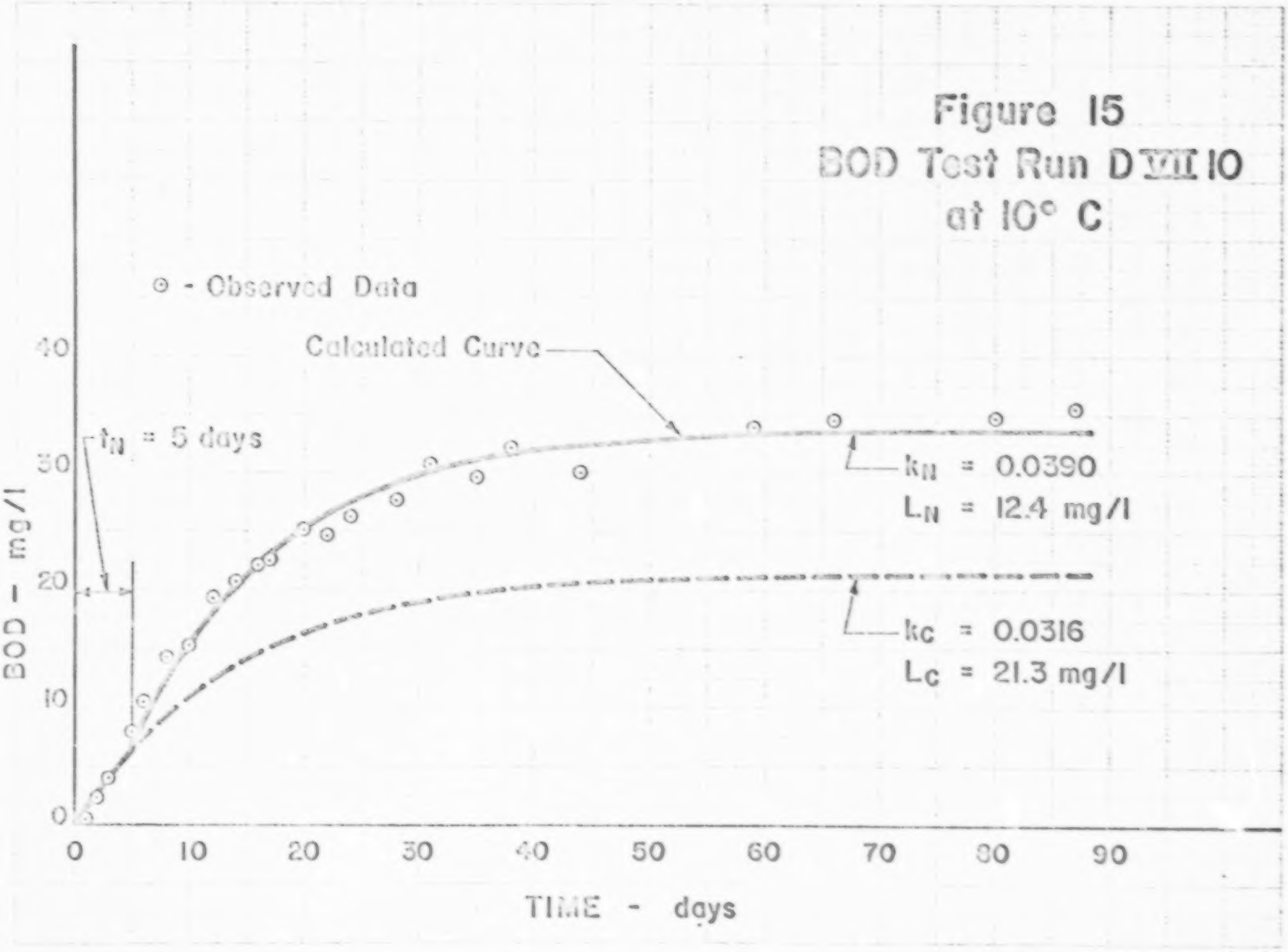

Figure 15
BOD Test Run D VII 10
at 10° C

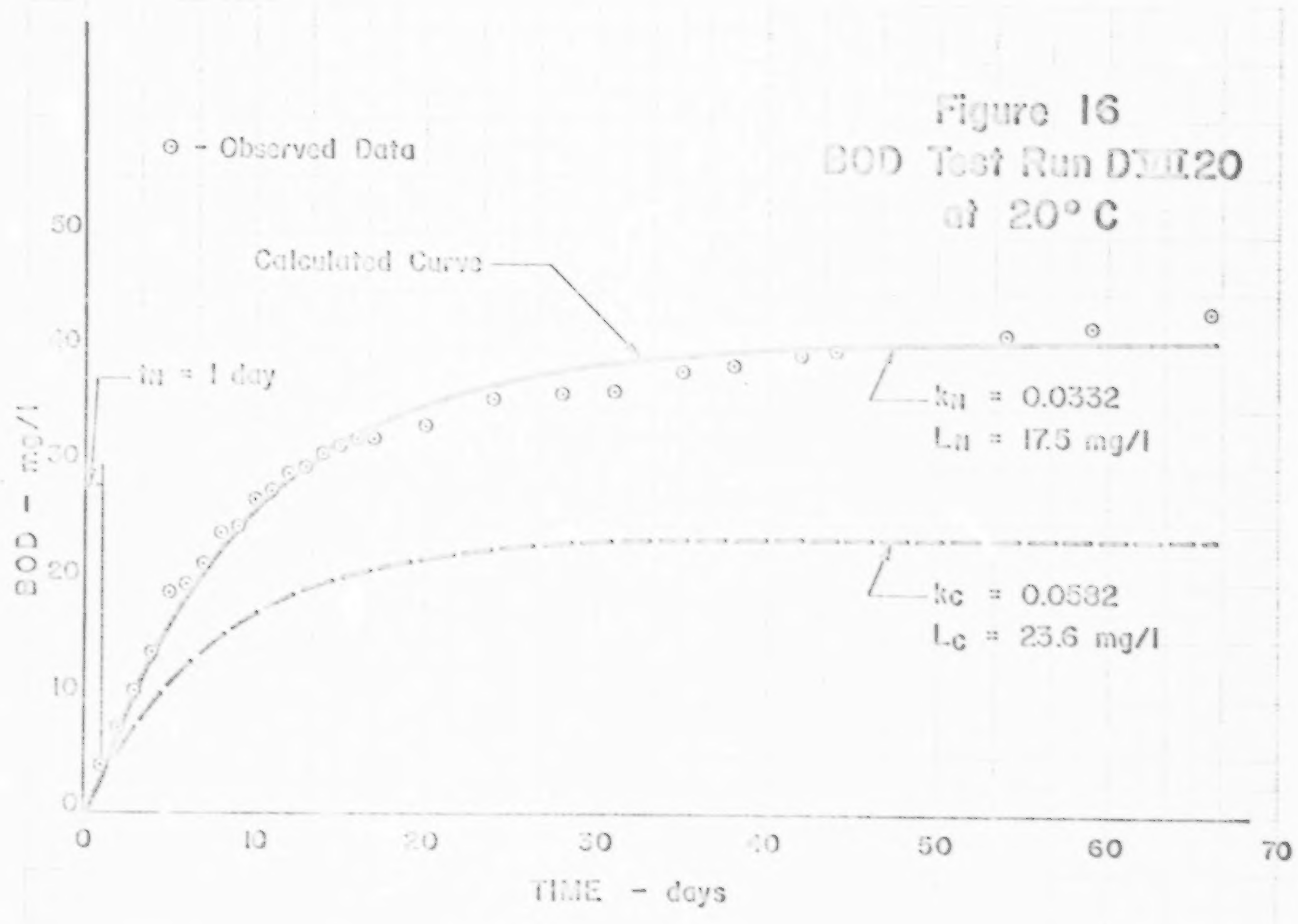

Figure 16
BOD Test Run DVIII20
at 20° C

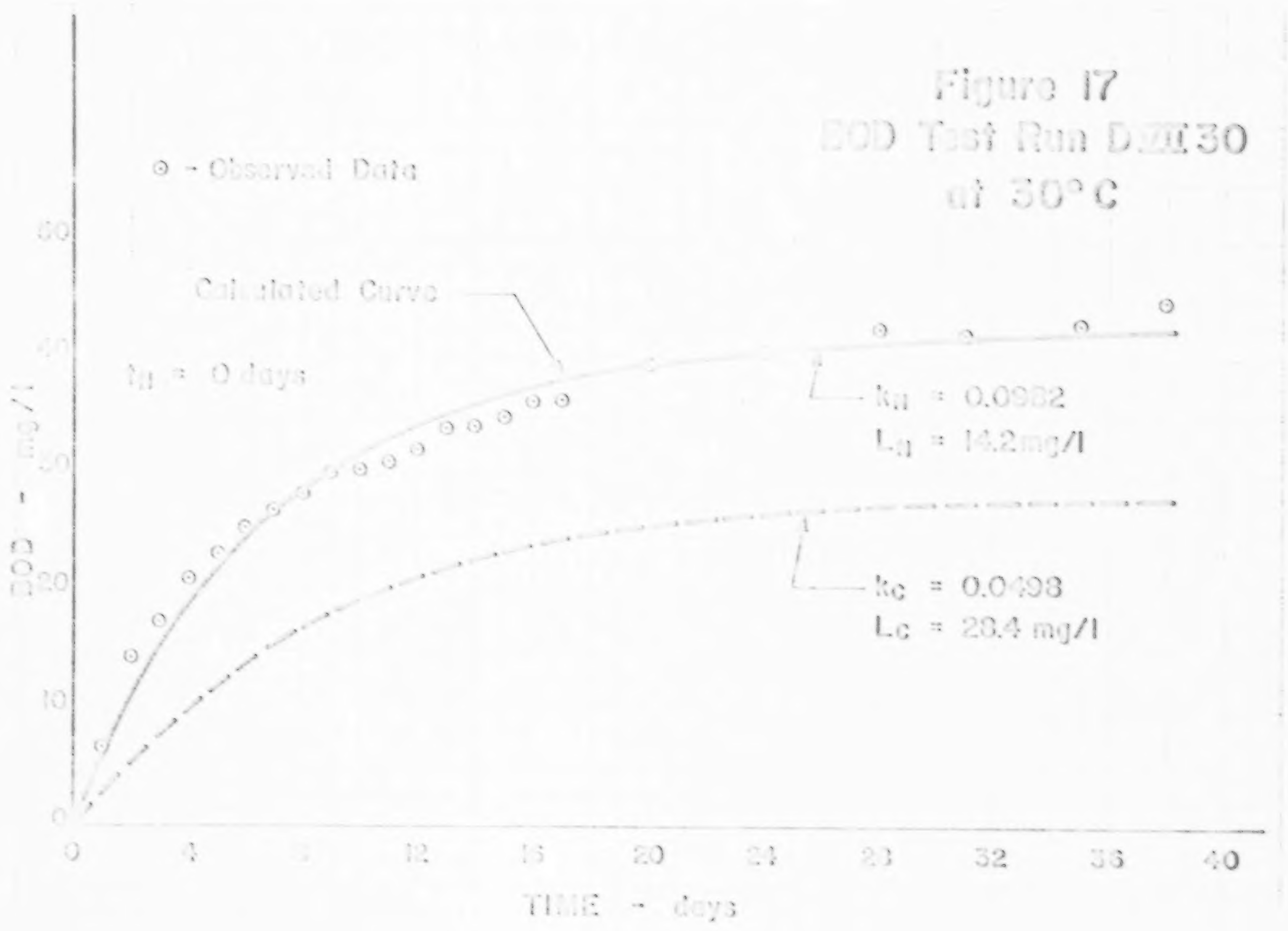

Figure 17
BOD Test Run D-XII 30
at 30°C

series. One important distinction between this test series and the others is in the characteristics of the effluent sample. The sum of the ultimate demands of this series was the lowest of all the series, indicating that a more stabilized effluent was probably being used. It would appear that as an effluent becomes increasingly more stable (or more highly treated), the two different types of deoxygenation become more competitive from a rate of reaction standpoint. In the case of the less stabilized effluents, the chemoorganotrophs which participate in the carbonaceous deoxygenation have a decided advantage from an activity point of view over the inherently slower nitrifiers. This test points out the importance of characterizing the type of substrate involved before generalizing on the deoxygenation behavior. Though, for the most part, the results of this test series were included in the final analysis of the data, certain atypical results were excluded when it was felt that they were completely out of line with the remaining results. These exceptions will be noted further on in the report.

5. Test Series E-XI

The results of the test series E-XI are presented in Figures 18 through 22. This is the first series which included five test runs set up on the same day. As noted previously, the results of the previous four series indicated

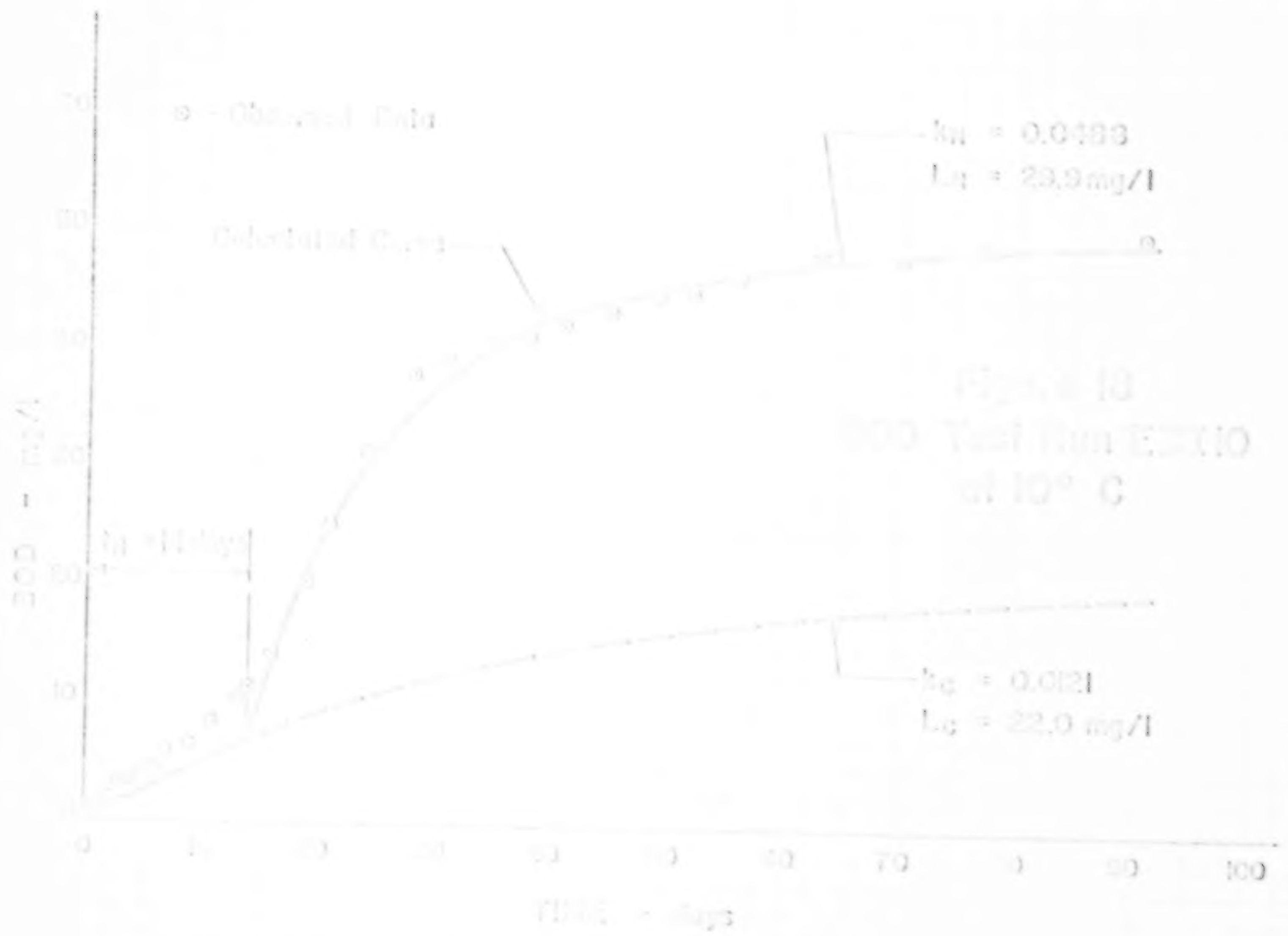

Figure 13
BOD Test Run E[illegible]10
at 10° C

57-a

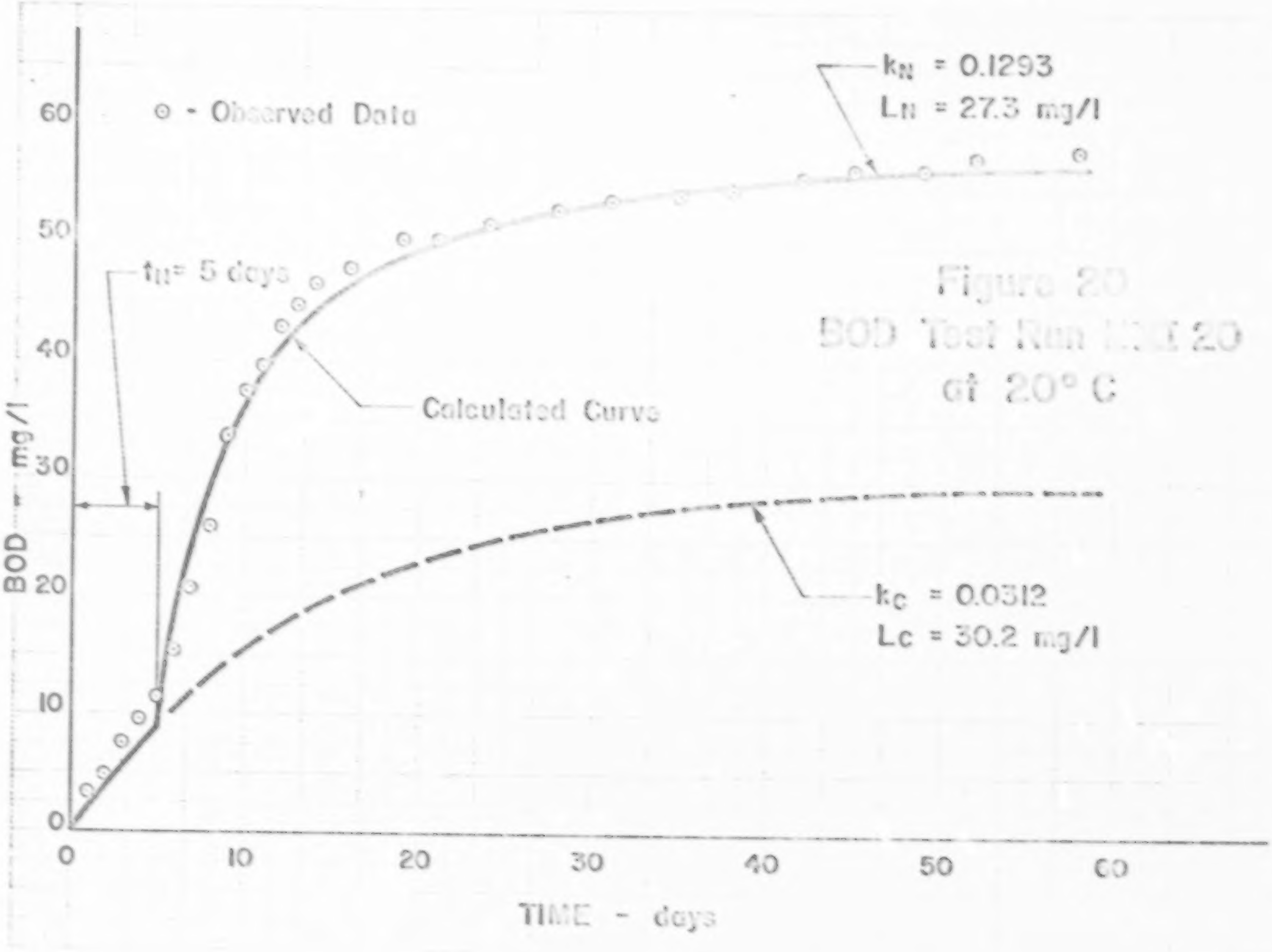

Figure 20
BOD Test Run [illegible] 20
at 20° C

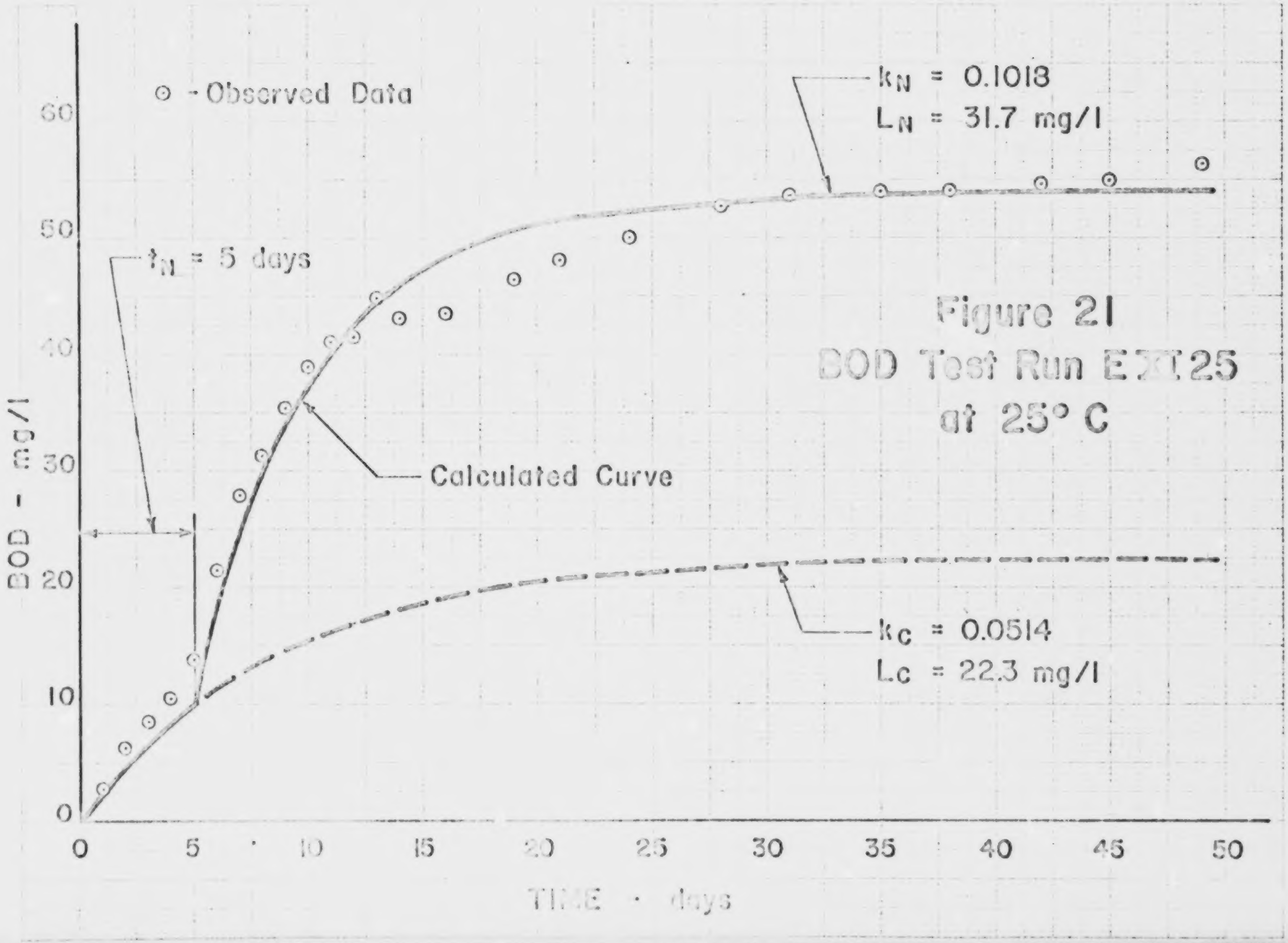

Figure 21
BOD Test Run EXI25
at 25° C

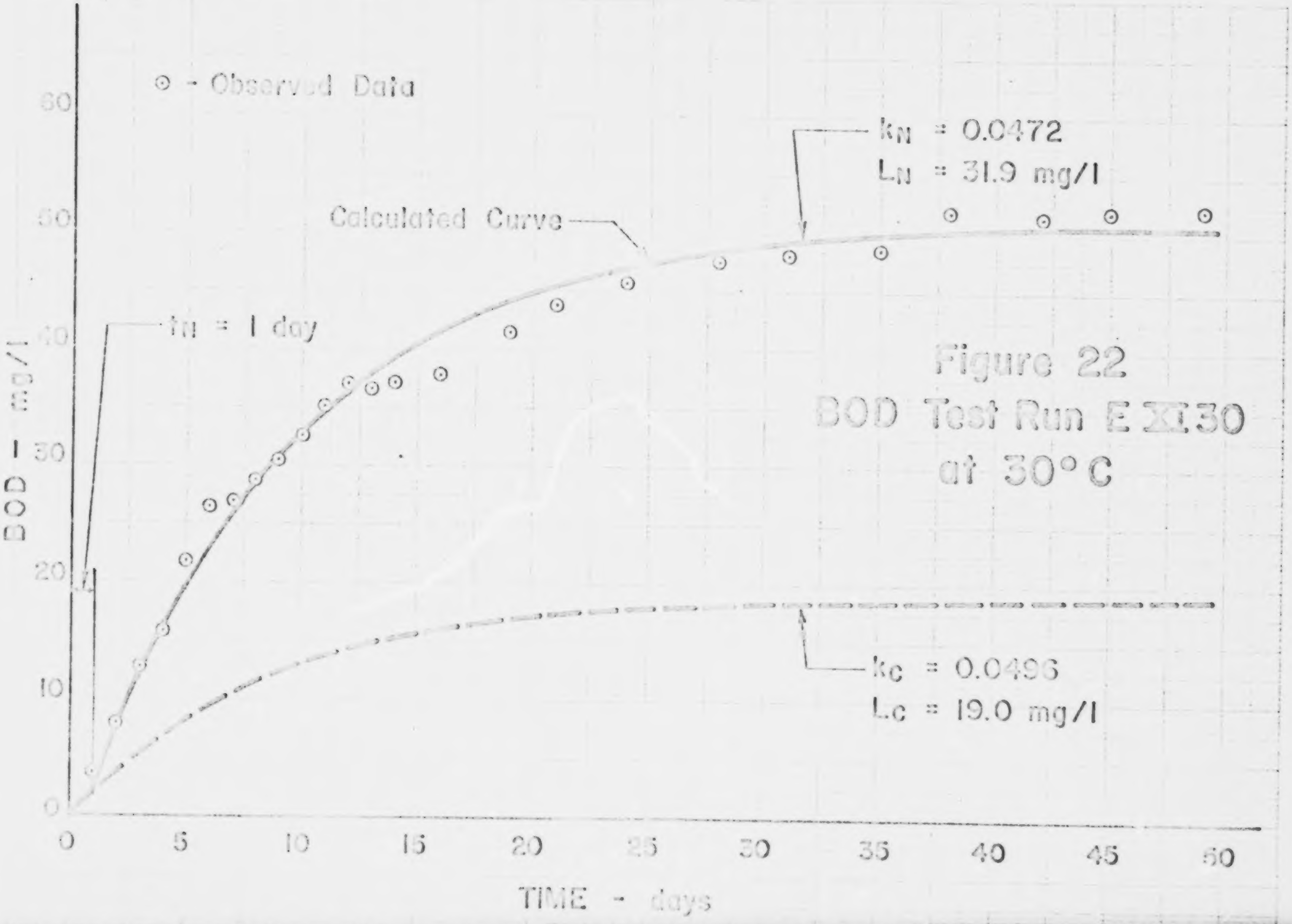

Figure 22
BOD Test Run E XI 30
at 30° C

that such an arrangement would most likely lead to the most fruitful results. As expected, the time to the nitrification onset decreased with the increasing incubation temperature. Both the 10°C and 15°C test runs exhibited a marked transitional range.

6. Test Series F-I

The results of test series F-I are shown in Figures 23 through 27. This series behaved in most respects like the previous test series. One difference is that both the 25°C and 30°C test runs exhibited some of the most erratic BOD results of all the other test runs, particularly in the later stages. The actual reason for this situation is not really known.

7. Test Series G-III

Test series G-III is different from the other series in that it exhibits the longest time periods to the nitrification onset. The results are presented in Figures 28 through 32. It is very unlikely that this is due to a difference in the seed, since all of the test runs responded identically from the standpoint of longer t_N values. It is more likely that some characteristics of the particular effluent sample caused this situation. One possibility is that an effluent less stable from a carbonaceous demand standpoint could extend this time period.

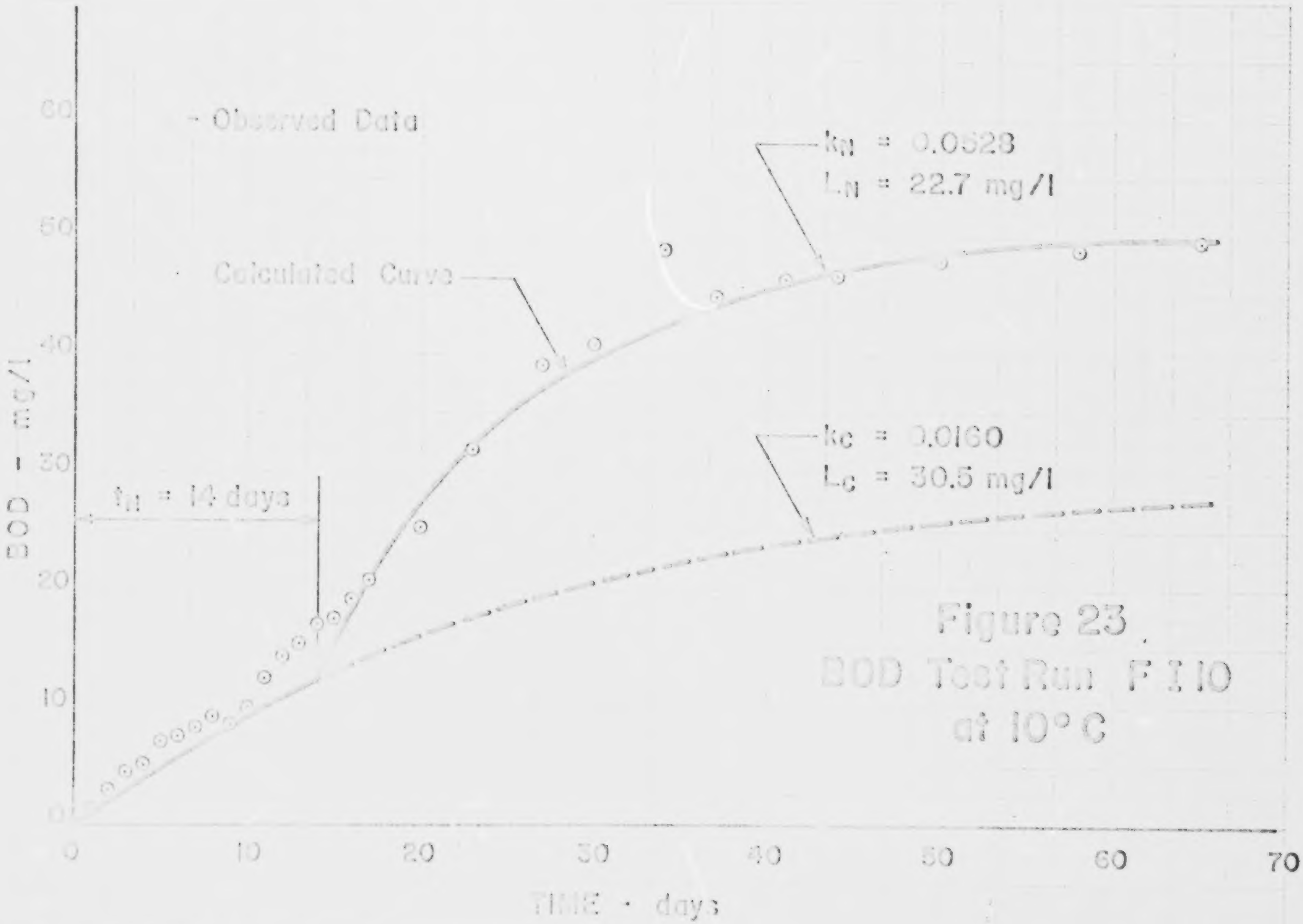

Figure 23
BOD Test Run F I 10
at 10° C

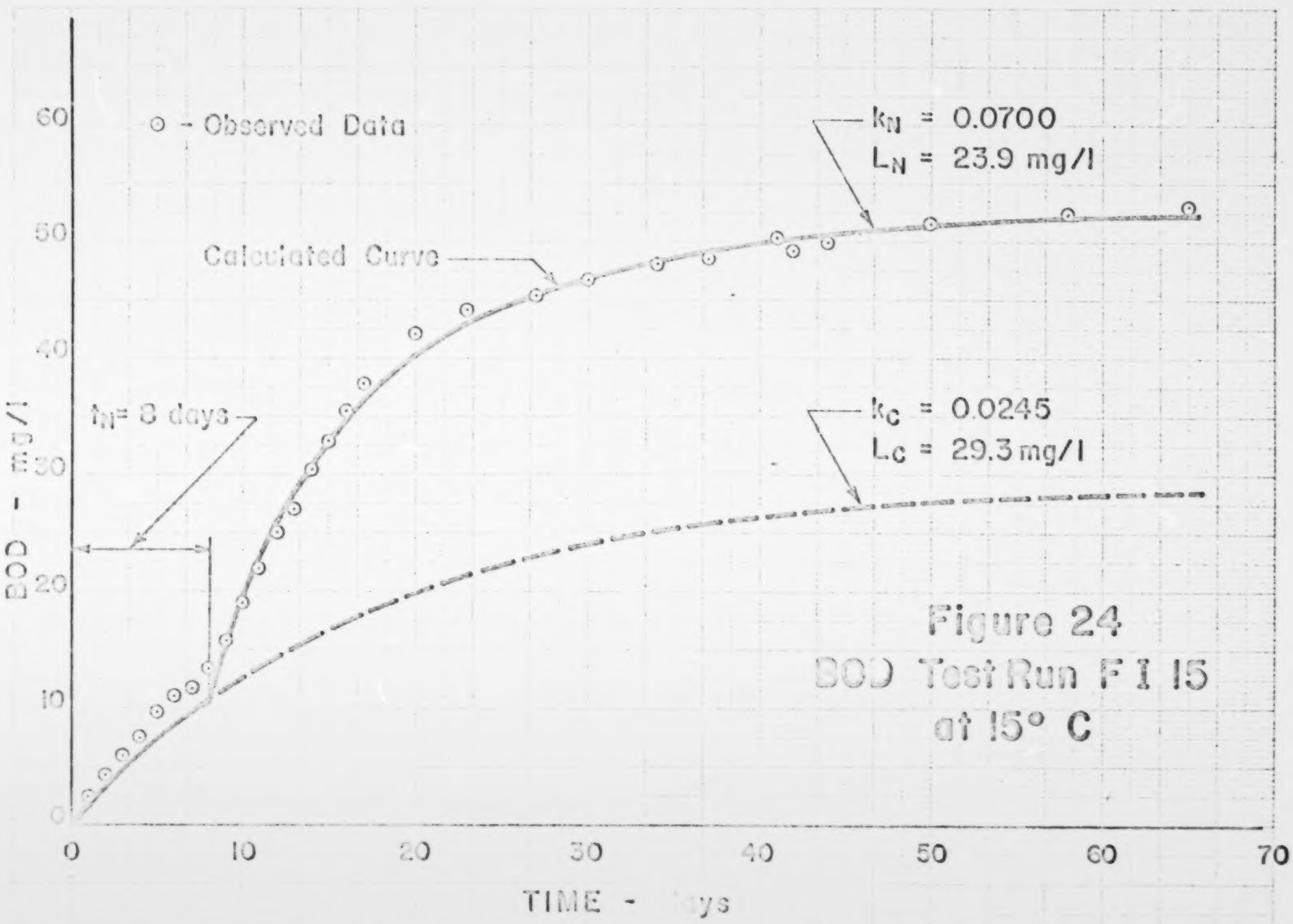

Figure 24
BOD Test Run F I 15
at 15° C

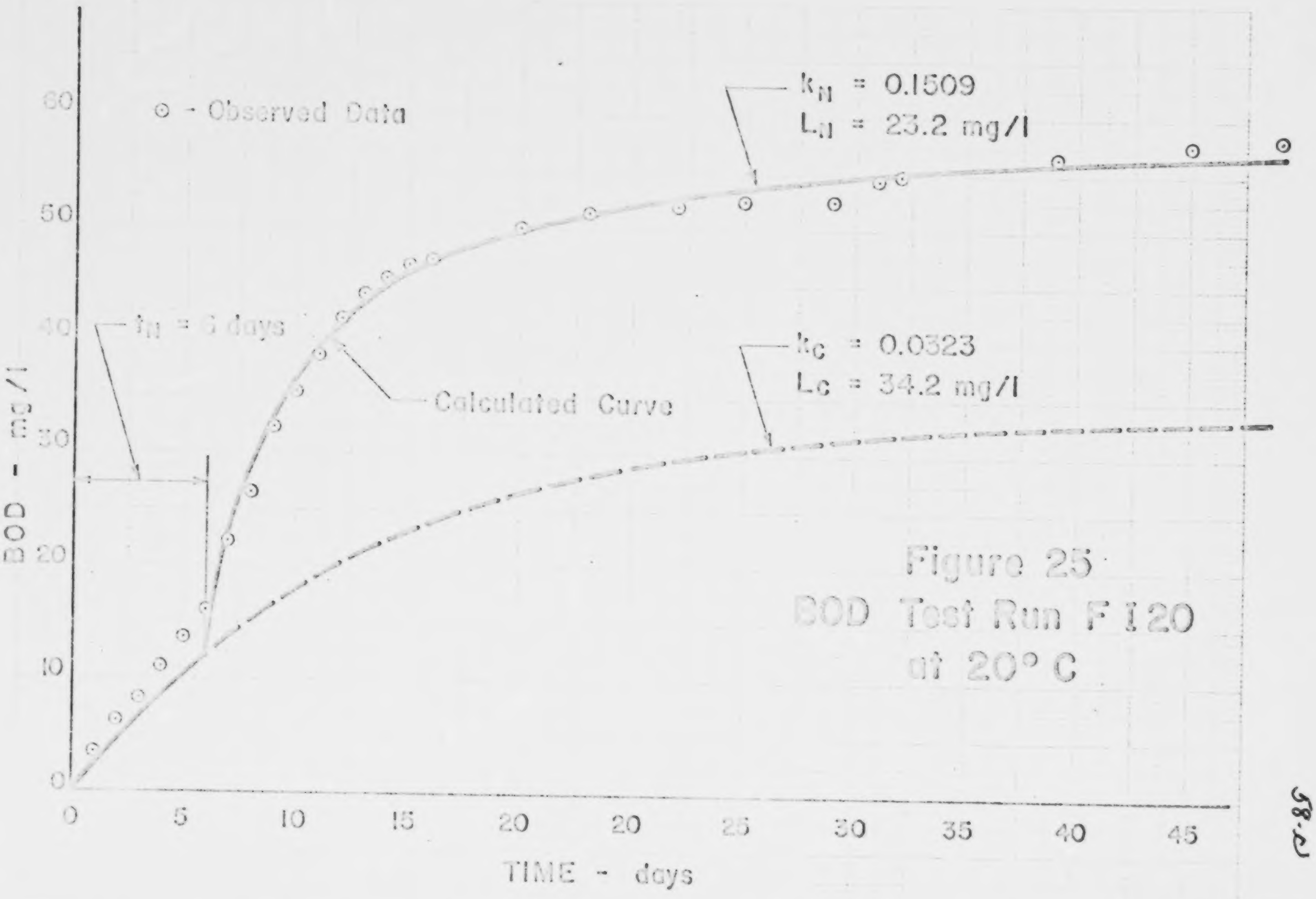

Figure 25
BOD Test Run F I20
at 20° C

26

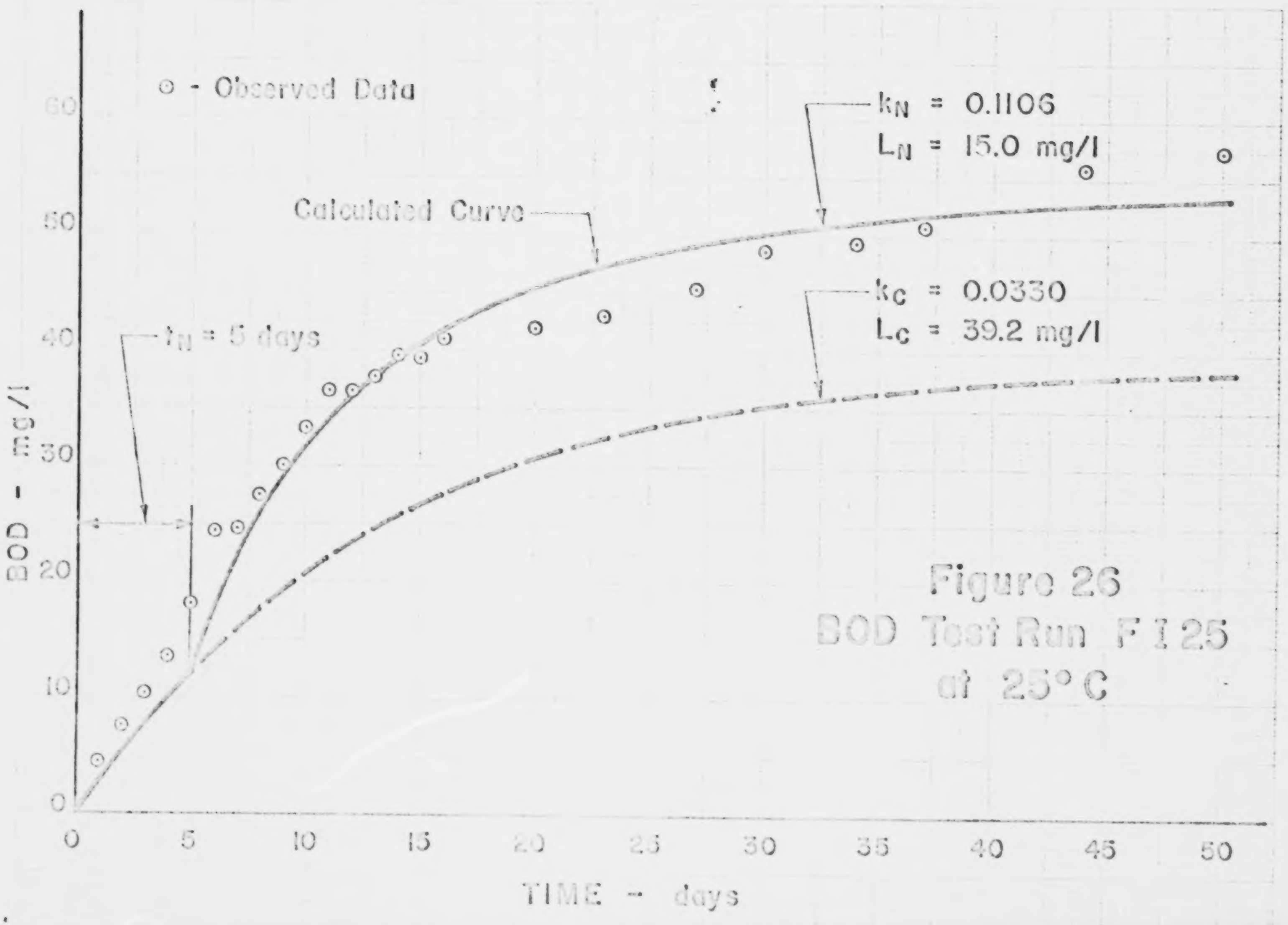

Figure 26
BOD Test Run F I 25
at 25° C

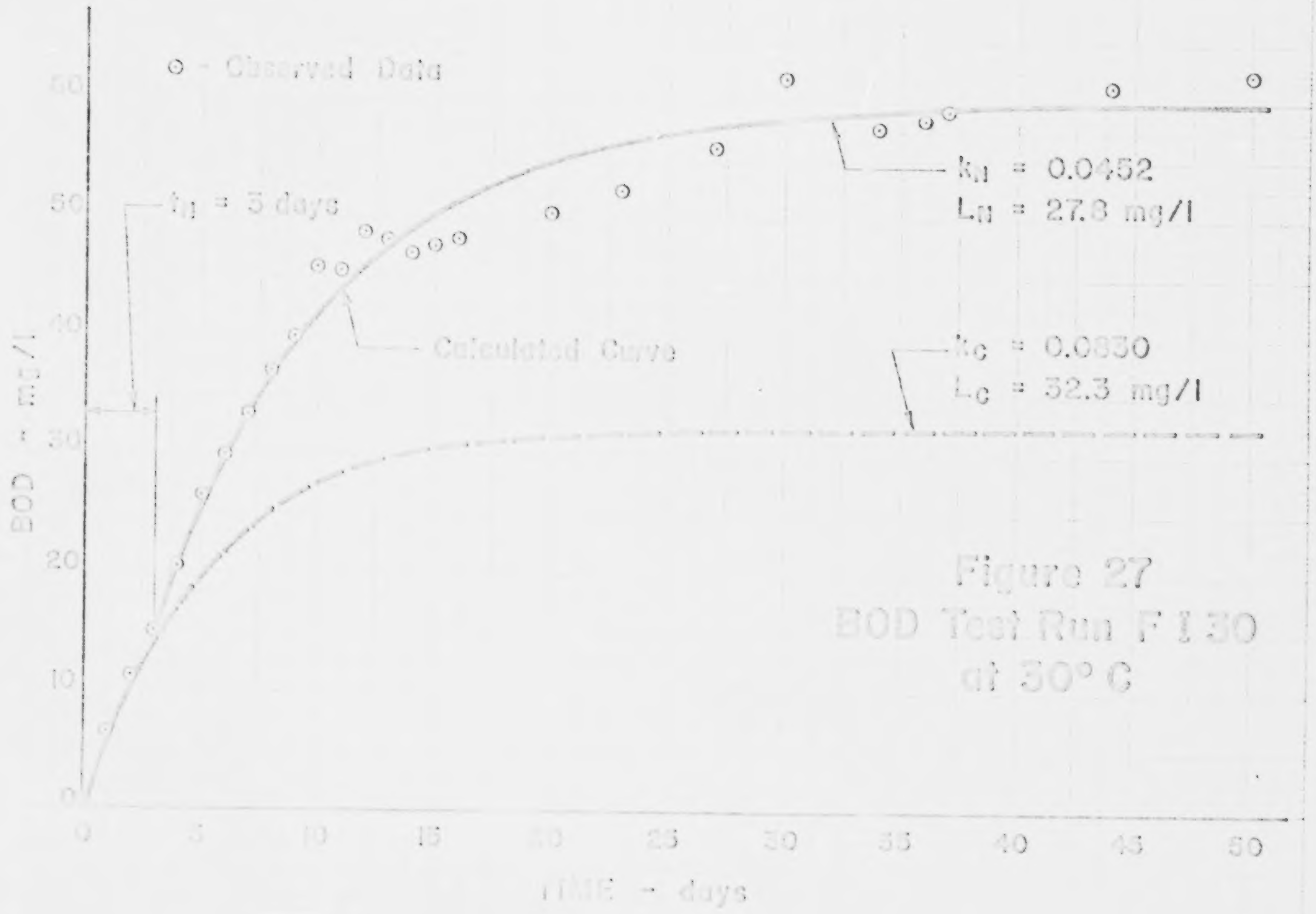

Figure 27
BOD Test Run F I 30
at 30° C

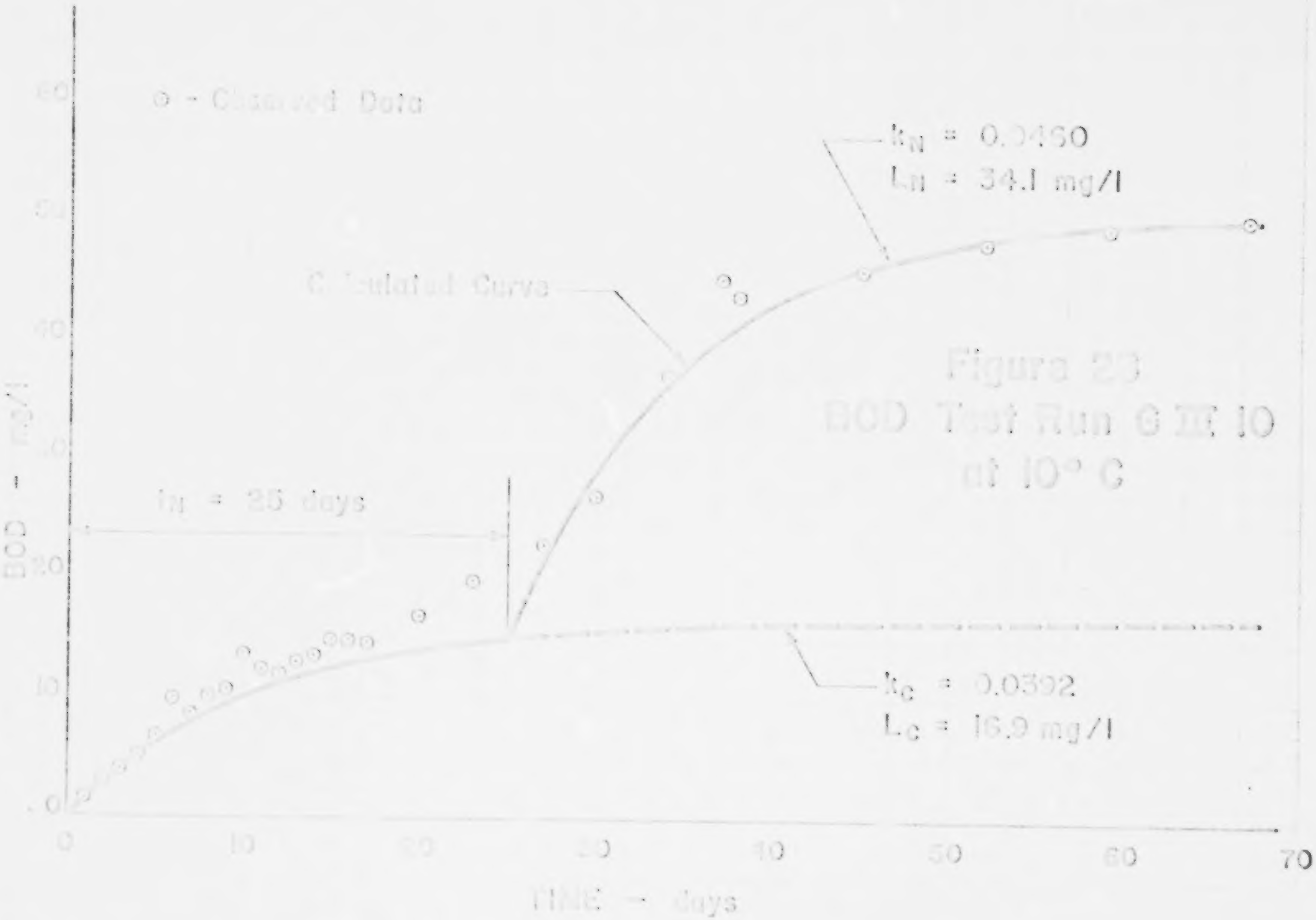

Figure 23
BOD Test Run G III 10
at 10° C

79

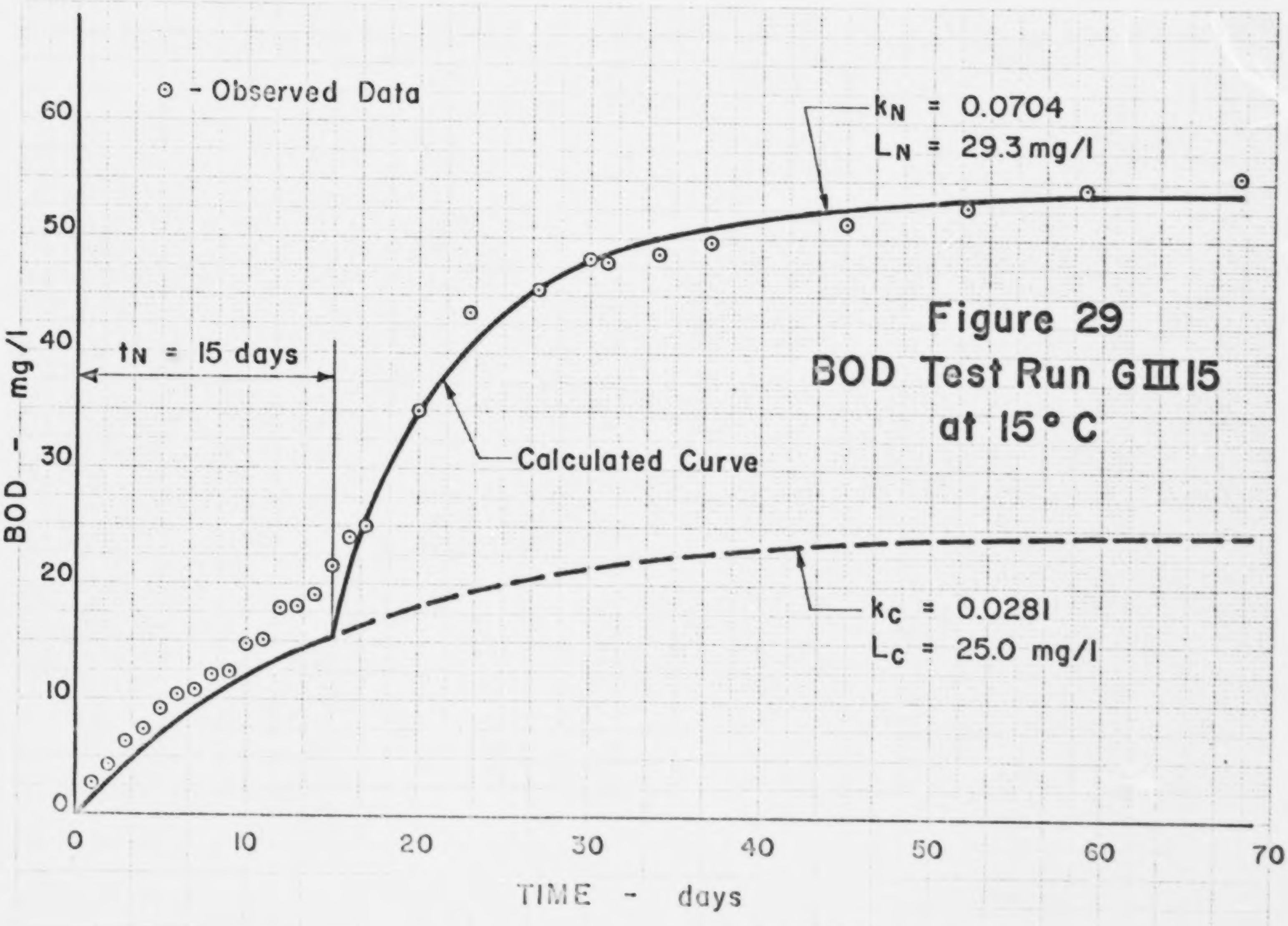

Figure 29
BOD Test Run GIII15
at 15° C

58-g

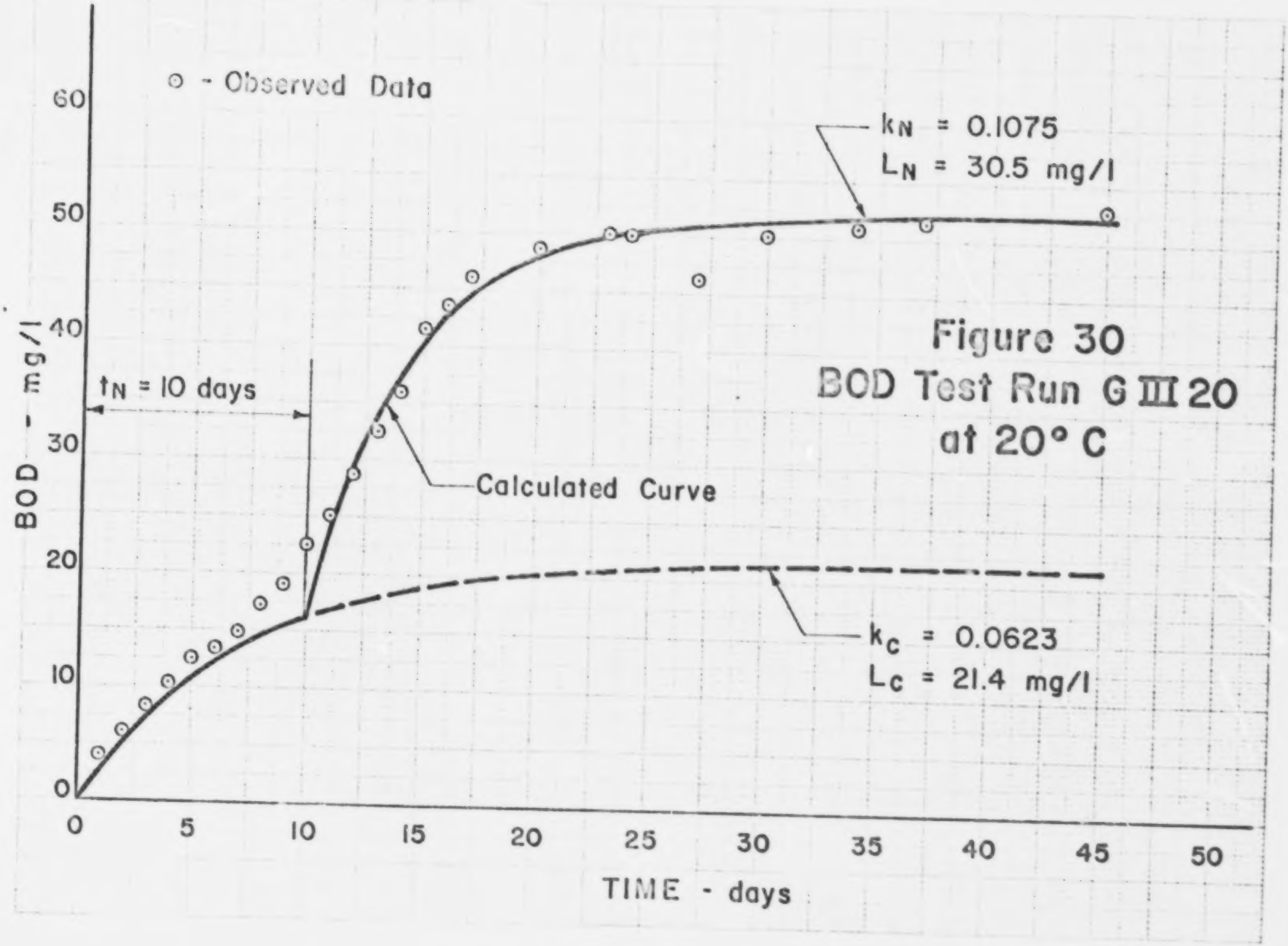

Figure 30
BOD Test Run G III 20
at 20° C

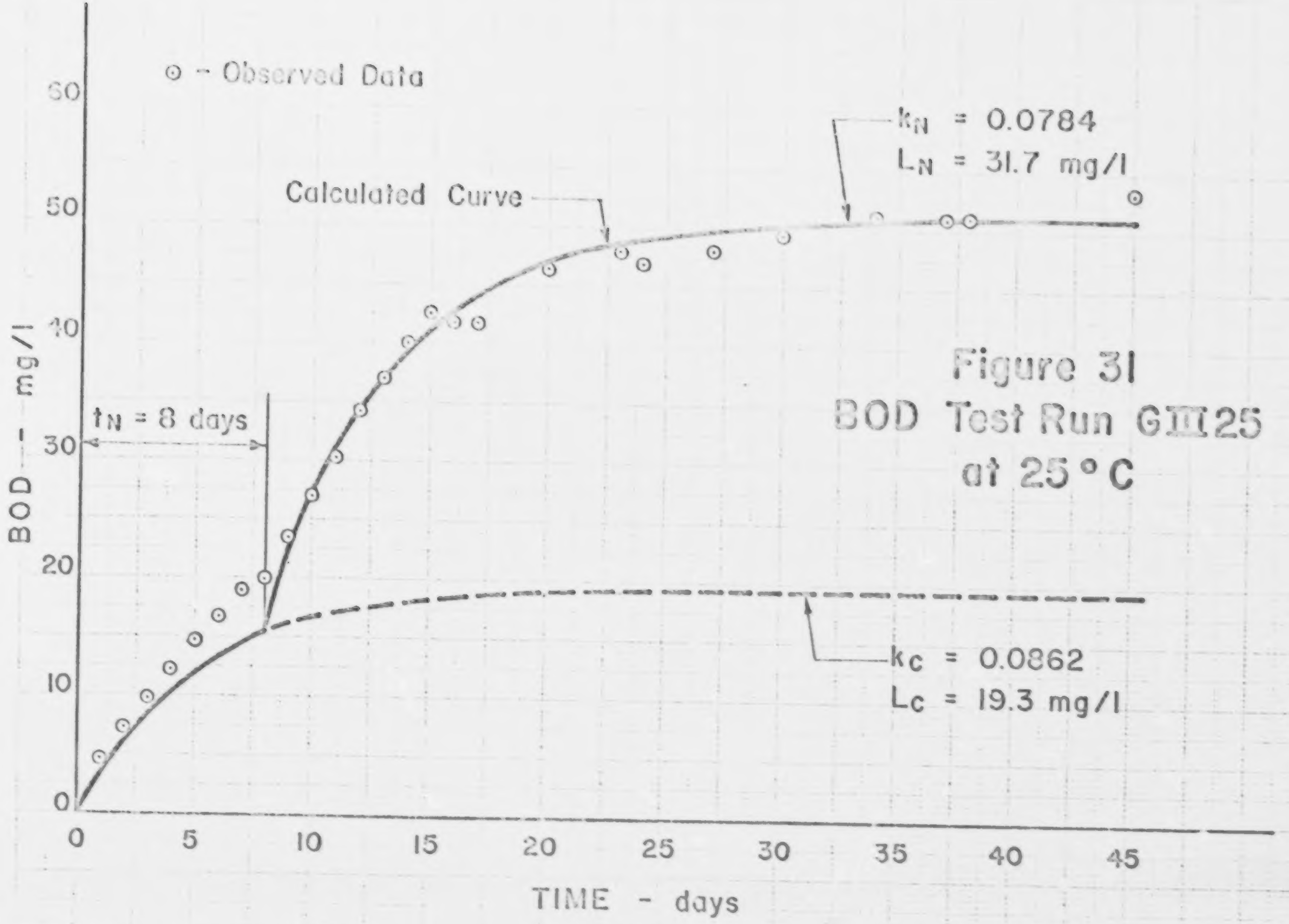

Figure 31
BOD Test Run GIII25
at 25 °C

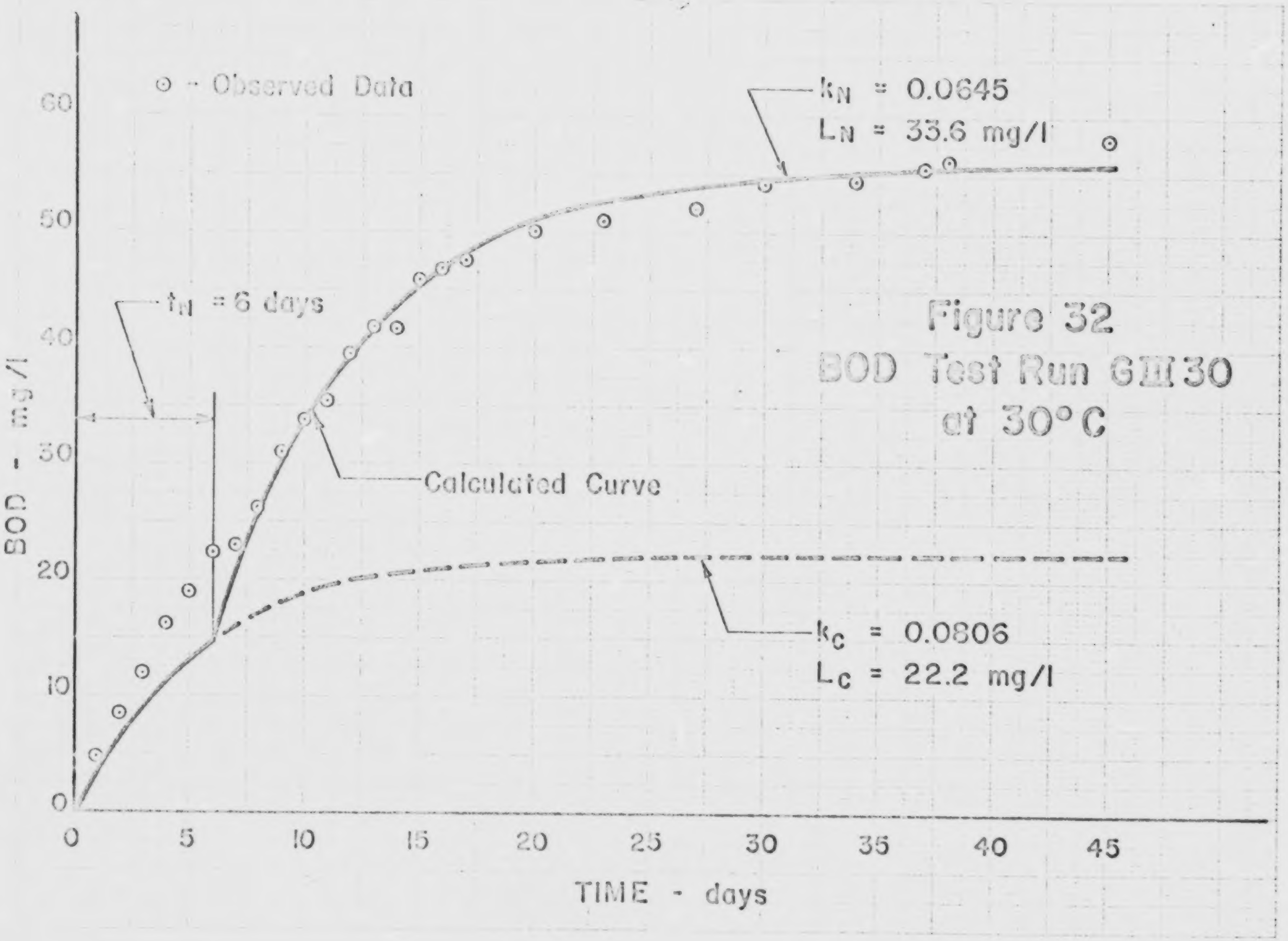

Figure 32
BOD Test Run GIII30
at 30°C

8. Test Series H-V

As mentioned previously, this test series included only nitrogen analyses, and was set up to determine as to what extent the seed quantity might influence the nitrification behavior. Some insight into this question can be gained by examining the nitrite-nitrogen production in this test series under four different seeding conditions. These plots are shown in Figures 33 and 34. Upon an examination of Figure 33, it would seem that the seed quantity had little effect on the nitrite-formation activity of the secondary effluents. Even the "no" seed condition was not markedly different from the highest seed condition. This observation is important since one can assume that the nitrification deoxygenation constants should not vary significantly with seed quantity. Figure 34 also demonstrates that there is no discernable relationship between the seed quantity and the nitrite production. Though the nitrate-nitrogen data were not plotted, an examination of these data presented in Appendix B would lead one to similar conclusions.

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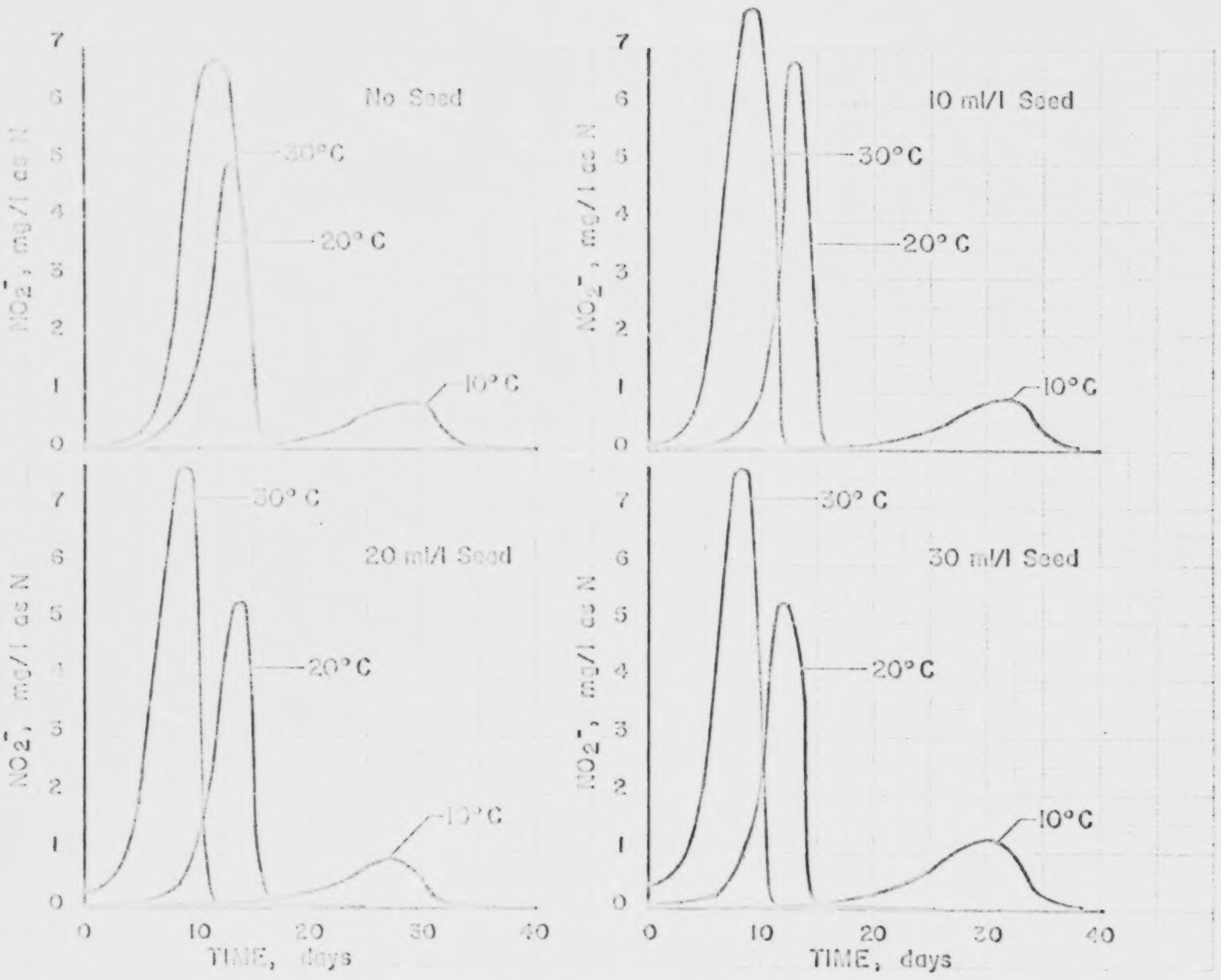
9. Test Series J-VI

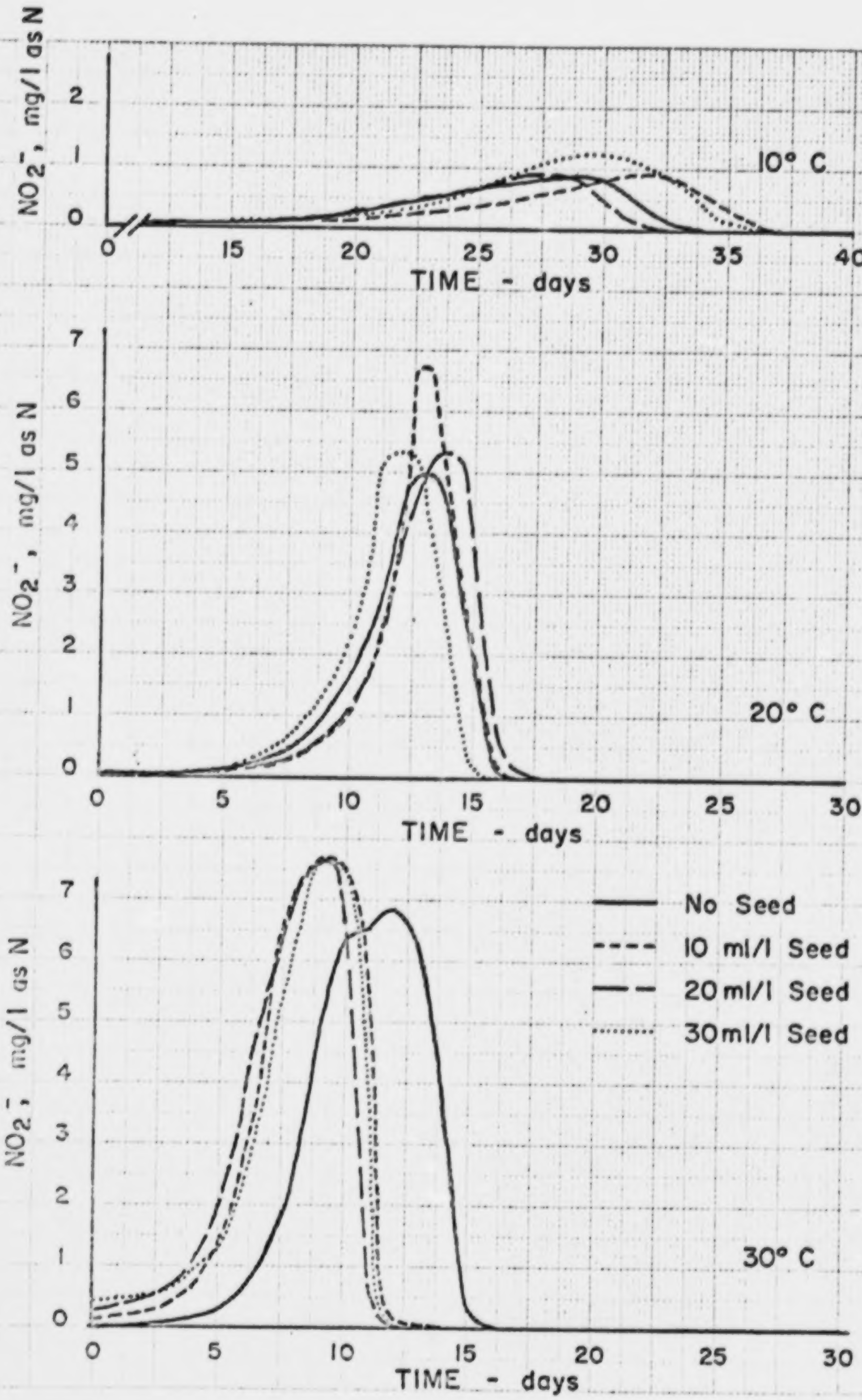
Test series J-VI was designed to determine as to what extent both the seed quantity, and the available ammonia-nitrogen influenced the nitrification activity. Some of the data are shown plotted in Figures 35, 36 and 37. Figure 35, which shows the relationship between the nitrite production



NO_2-N Formation With Varying Seed Quantities--Temperatures 10, 20 & 30°C

FIGURE 33

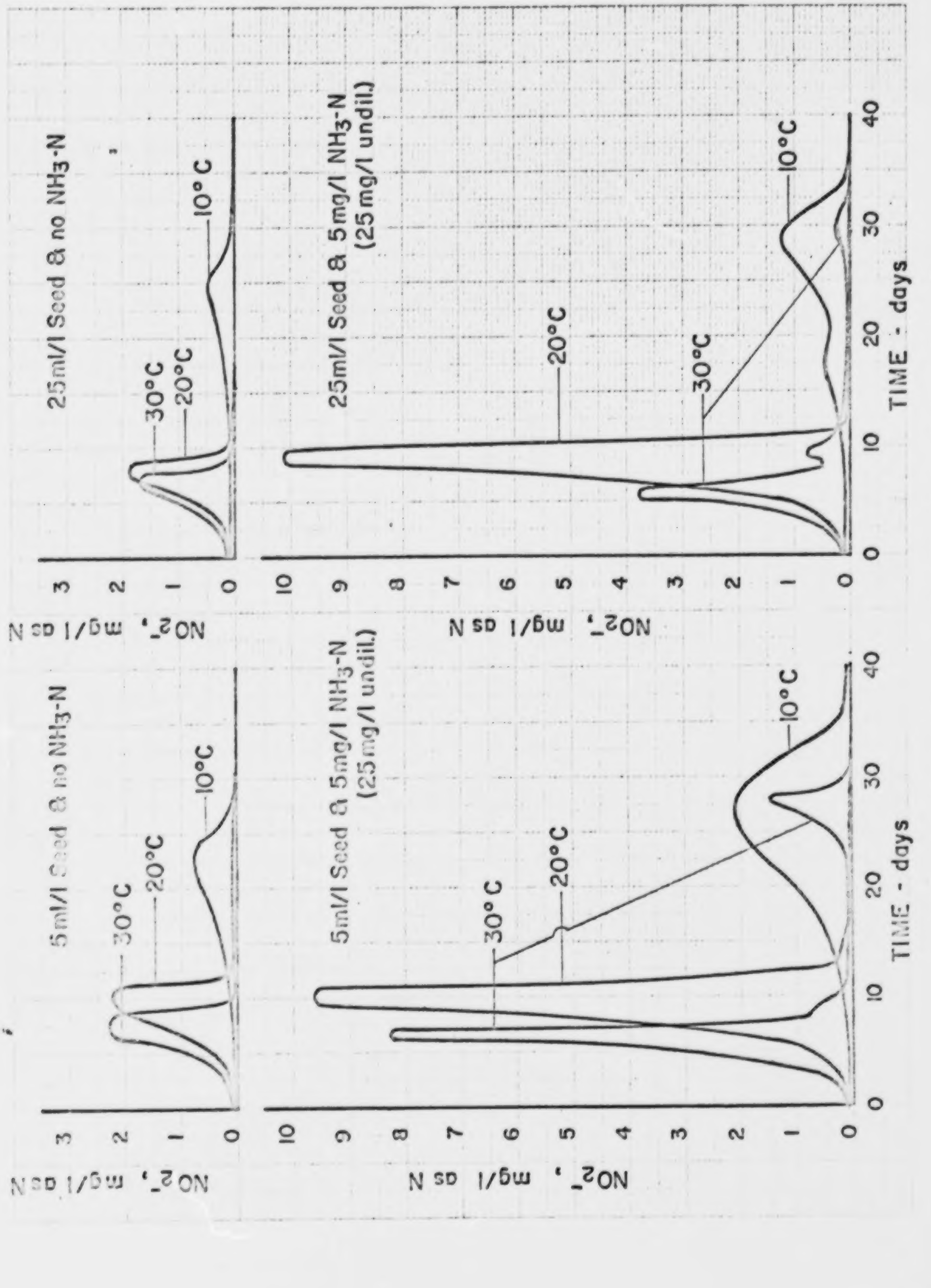
59-b



NO$_2$-N. Formation at 10, 20 & 30°C--Seed Concentrations
0, 10, 20 and 30 ml/l

FIGURE 34

59-a



NO_2-N Formation with Varying Seed and NH_3-N Quantities-- Temperatures 10, 20 & 30°C

FIGURE 35

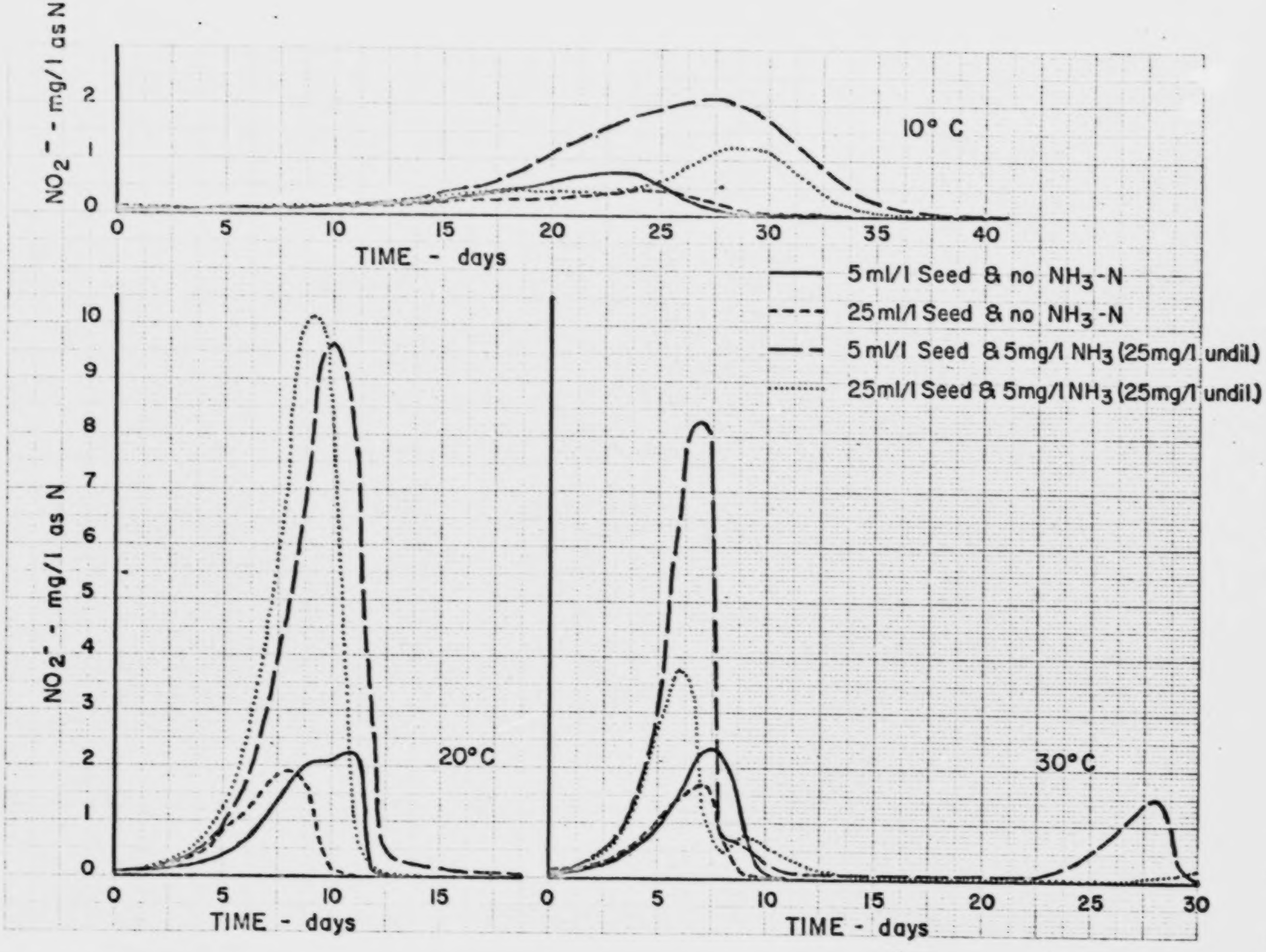
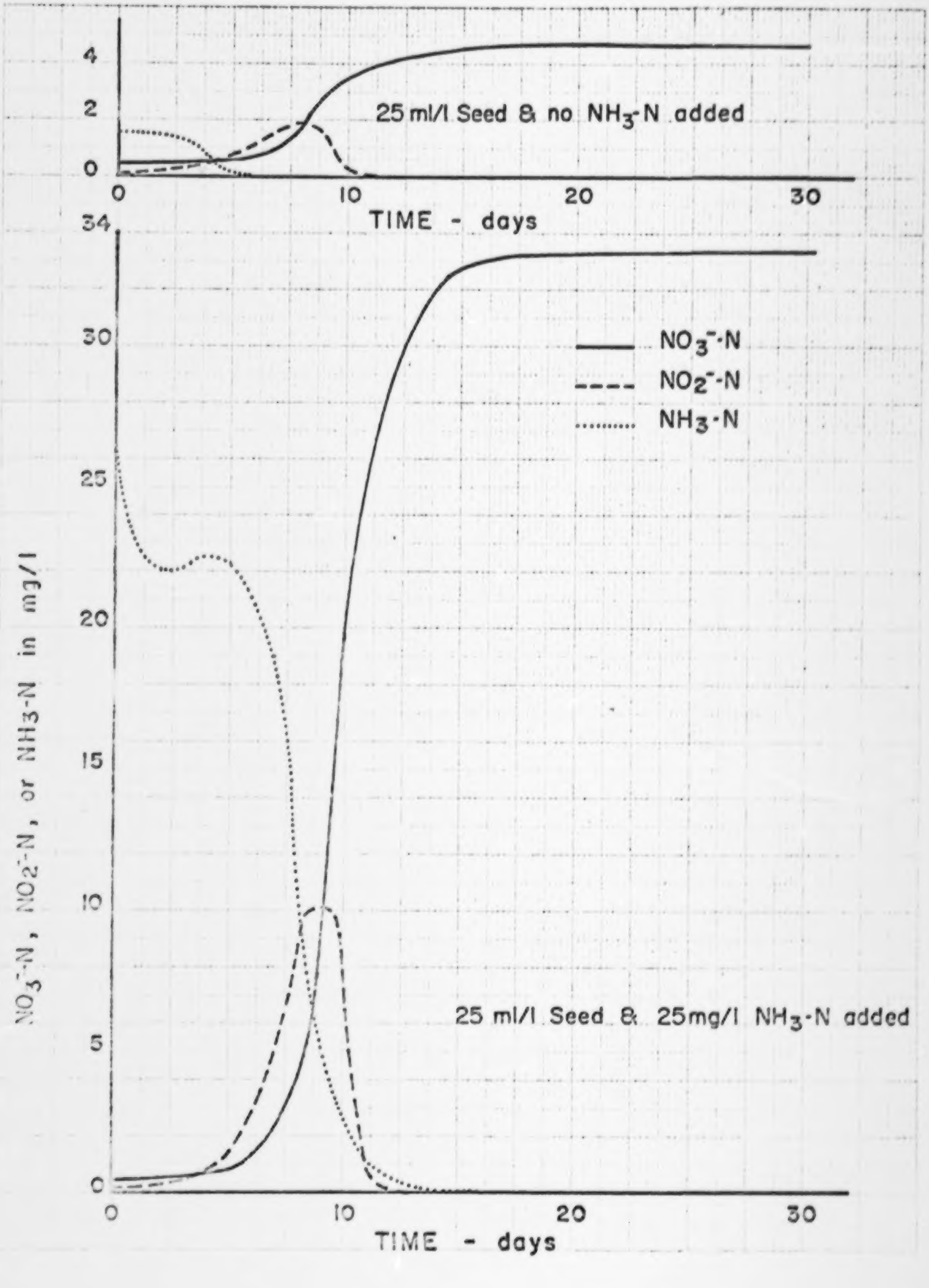


FIGURE 36

NO$_2$-N Formation at 10, 20 & 30°C with Varying Seed and NH$_3$-N Quantities

59-2



Relationship Between NO_2, NO_3 and NH_3-N at 20°C with and Without NH_3-N Added

FIGURE 37

and the two variables mentioned above, demonstrates for the most part that the seed quantity has little effect. The plots with and without ammonia have the same pattern except that the test runs with ammonia exhibited a much higher peak. It is interesting that this peak reached a magnitude of approximately 10 mg/l, in spite of the fact that 25 mg/l of ammonia nitrogen was available. This indicates that the nitrite production simulates the development of nitrate-forming organisms, which eventually oxidizes the nitrite ion to the nitrate form.

Another interesting situation shown in Figure 35 is the secondary rise in nitrite production, which occurs in the later stages of deoxygenation, in the case of the test runs with ammonia nitrogen added. This same condition was exhibited in many of the test runs but to a much smaller degree. The large rise in this case was probably due to the higher nitrogen levels. One possible explanation for this secondary rise is the oxidation of more resistant organics, which takes place only after extended incubation periods. For example, these organics may have originated from heterotrophic bacteria or possibly protozoa, which eventually die because of the depletion of the food supply.

Figure 36 shows the same data, only comparing the four set-up conditions at each temperature. It is interesting to note that at 30°C, the high seed set-up had a lower nitrite peak than the low seed set-up, comparing the two set-ups in which ammonia was added. This indicates that possibly the higher seed content included also more nitrate-formers which responded quickly at this warmer temperature.

Figure 37 shows the relationship between the various forms of nitrogen, with and without the added ammonia nitrogen. The patterns were very similar for the two different ammonia levels. In both cases the nitrite-nitrogen and nitrate-nitrogen peaks occurred at about the same time. The ammonia-nitrogen was depleted sooner when none was added which is to be expected. These results indicated that with sufficient seed present, there is little difference in the nitrification behavior with different levels of ammonia-nitrogen present.

D. Summary of Deoxygenation Results

The velocity constants and ultimate demands of all the test runs, for both the carbonaceous and nitrogenous stages of deoxygenation, shown in the previous figures, will be presented in a summary form in the following sections. The tables include both individual values and average values for the respective incubation temperatures. Since 20°C test runs were included in all of the test series, individual test run values of k, L and t_N were also expressed relative to the 20°C value. By expressing the results in this manner, it is felt that the results of the different series can be more realistically compared, since the changes in the characteristics of the test substrate makes it difficult to logically compare the absolute values obtained for the different series. Expressing the results in the form of these ratios will then be advantageously employed for developing the relationship between the individual test run results and the temperature later on in the report.

1. Carbonaceous Deoxygenation

A summary of the carbonaceous deoxygenation constants is presented in Table 7. The average value of the velocity constant increases with increasing temperature which is to be expected, though significant variations do occur at each incubation temperature. The constants expressed in a ratio form exhibit much less variation from test run to test run.

2. Nitrogenous Deoxygenation

The nitrogenous deoxygenation constants are presented in Table 8. Again the average velocity constant increases with increasing temperature except at 35°C. Interestingly, the average values of the nitrogenous velocity constant are greater than those of the carbonaceous stage, at all the incubation temperatures. Also, the nitrogenous values exhibited less variation from test run to test run.

3. Relationship Between the Two Deoxygenation Stages

The relationship between the carbonaceous and the nitrogenous deoxygenation activities is well demonstrated

TABLE 7

Carbonaceous Deoxygenation Constants

		Carbonaceous Deoxygenation Constants			Ratio of Constant to 20°C Value Same Series			
		Each Test Run		Average for T	Each Test Run		Average for T	
Test Run	Temp. (T) °C	k(day^{-1})	L(mg/l)	k(day^{-1})	k_T/k_{20}	L_T/L_{20}	k_T/k_{20}	L_T/L_{20}
B-IV	5	0.0119	37.9	0.0119	0.204	0.907	0.204	0.907
A-II	10	0.0190	34.4	0.0236	0.605	0.827	0.514	0.828
D-VII	10	0.0316	21.3		0.543	0.903		
E-XI	10	0.0121	22.0		0.387	0.730		
F-I	10	0.0160	30.5		0.494	0.892		
G-III	10	0.0392	16.9		0.629	0.788		
C-VI	15	0.0585	36.8	0.0323	0.769	1.179	0.639	1.030
E-XI	15	0.0180	27.6		0.579	0.915		
F-I	15	0.0245	29.3		0.759	0.858		
G-III	15	0.0281	25.0		0.452	1.167		
A-II	20	0.0314	41.6	0.0500				
B-IV	20	0.0584	41.8					
C-VI	20	0.0761	31.2					
D-VII	20	0.0582	23.6					
E-XI	20	0.0312	30.2					
F-I	20	0.0323	34.2					
G-III	20	0.0623	21.4					
C-VI	25	0.0772	31.0	0.0620	1.014	0.994	1.267	0.945
E-XI	25	0.0514	22.3		1.650	0.739		
F-I	25	0.0330	39.2		1.022	1.147		
G-III	25	0.0862	19.3		1.384	0.900		
A-II	30	0.0542	36.8	0.0634	1.726	0.885	1.607	0.940
D-VII	30	0.0498	28.4		0.856	1.203		
E-XI	30	0.0496	19.0		1.591	0.631		
F-I	30	0.0830	32.3		2.567	0.947		
G-III	30	0.0806	22.2		1.294	1.035		
B-IV	35	0.0570	51.2	0.0570	0.976	1.225	0.976	1.225

TABLE 8

Nitrogenous Deoxygenation Constants

		Nitrogenous Deoxygenation Constant			Ratio of Constant to 20°C Value Same Series			
		Each Test Run		Average for T	Each Test Run		Average for T	
Test Run	Temp. (T) °C	k(day^{-1})	L(mg/l)	k(day^{-1})	k_T/k_{20}	L_T/L_{20}	k_T/k_{20}	L_T/L_{20}
B-IV	5	0.0271	20.9	0.0271	0.236	0.959	0.236	0.959
A-II	10	0.0786	30.8	0.0530	0.492	1.034	0.412	0.987
D-VII	10	0.0390	12.4		1.175*	0.709		
E-XI	10	0.0488	29.9		0.377	1.095		
F-I	10	0.0528	22.7		0.350	0.978		
G-III	10	0.0460	34.1		0.428	1.119		
C-VI	15	0.0785	37.2	0.0730	0.736	0.979	0.606	1.021
E-XI	15	0.0732	30.4		0.566	1.114		
F-I	15	0.0700	23.9		0.464	1.029		
G-III	15	0.0704	29.3		0.655	0.961		
A-II	20	0.1600	29.8	0.1282			1.000	1.000
B-IV	20	0.1149	21.8					
C-VI	20	0.1066	38.0					
D-VII	20	0.0332*	17.5					
E-XI	20	0.1293	27.3					
F-I	20	0.1509	23.2					
G-III	20	0.1075	30.5					
C-VI	25	0.0961	36.9	0.0967	0.902	0.971	0.788	0.955
E-XI	25	0.1018	31.7		0.788	1.161		
F-I	25	0.1106	15.0		0.733	0.648		
G-III	25	0.0784	31.7		0.729	1.041		
A-II	30	0.0795	31.8	0.0669	0.497	1.067	0.440	1.069
D-VII	30	0.0982	14.2		2.956*	0.811		
E-XI	30	0.0472	31.9		0.365	1.168		
F-I	30	0.0452	27.8		0.300	1.198		
G-III	30	0.0645	33.6		0.600	1.103		
B-IV	35	0.0660	20.0	0.0660	0.574	0.917	0.574	0.917

*Value excluded in calculation of average value in Tables 9 and 10. Table 9 presents a summary of the calculated ultimate demands for the two stages, grouped according to test series rather than the temperature as before. The variation in the values of the ultimate demand for each test series is particularly interesting since it is recalled that all of the test runs of a series were set up with the same effluent sample, and at the same time. There appears to be very little variation in the ultimate demands of both stages within each series. Also the fact that the ultimate demands did not vary to a great extent from series to series indicates that the effluent sample was rather consistent throughout the investigation. This situation lends a degree of confidence to the operation of using the different series to arrive at a single relationship between deoxygenation and temperature.

Another ratio of interest shown in this Table is that of the calculated nitrogenous ultimate demand to the carbonaceous ultimate demand. Such a parameter could conceivably be used to charactefize, in an approximate manner, the properties of the effluent sample being used. This parameter could serve as an aid in comparing the results of this investigation with those of others. Though the ratio varies somewhat, the average value for this investigation was 0.92, meaning that the two demands were nearly equal.

63-a

TABLE 9

Comparison of Carbonaceous and Nitrogenous Ultimate Demands by Series

Test Run	Temp. °C	Ultimate Demand (L) Carbonaceous mg/1 L_C	 Average	 Nitrogenous mg/l L_N	 Average	Ratio of Ultimate Demands L_N/L_C
A-II	10	34.4		30.8		
A-II	20	41.6	37.6	29.8	20.8	.82
A-II	30	36.8		31.8		
B-IV	5	37.9		20.9		
B-IV	20	41.8	43.6	21.8	20.9	.48
B-IV	35	51.2		20.0		
C-VI	15	36.8		37.2		
C-VI	20	31.2	33.0	38.0	37.4	1.13
C-VI	25	31.0		36.9		
D-VII	10	21.3		12.4		
D-VII	20	23.6	24.4	17.5	14.7	.60
D-VII	30	28.4		14.2		
E-XI	10	22.0		29.9		
E-XI	15	27.6		30.4		
E-XI	20	30.2	24.2	27.3	30.2	1.25
E-XI	25	22.3		31.7		
E-XI	30	19.0		31.9		
F-I	10	30.5		22.7		
F-I	15	29.3		23.9		
F-I	20	34.2	33.1	23.2	22.5	.68
F-I	25	39.2		15.0		
F-I	30	32.3		27.8		
G-III	10	16.9		34.1		
G-III	15	25.0		29.3		
G-III	20	21.4	21.0	30.5	31.8	1.51
G-III	25	19.3		31.7		
G-III	30	22.2		33.6		

TABLE 10

Relationship Between Carbonaceous and Nitrogenous Deoxygenation Stages

Test	Temp.	Nitrification Lag (t_N)				Velocity Constant Ratios	
		Number of Days		Ratio			
Run	°C	t_N	Avg.	$t_N/t_{N_{20}}$	Avg.	k_N/k_C	Average
B-IV	5	71	71	6.45	6.45	2.28	2.28
A-II	10	28	17.2	2.56	2.55	4.14	2.78
D-VII	10	5		5.00*		1.23	
E-XI	10	14		2.80		4.03	
F-I	10	14		2.33		3.30	
G-III	10	25		2.50		1.17	
C-VI	15	12	11.0	1.50	1.53	1.34	2.69
E-XI	15	9		1.80		4.07	
F-I	15	8		1.33		2.86	
G-III	15	15		1.50		2.50	
A-II	20	11	7.4		1.00	5.10	2.80
B-IV	20	11				1.97	
C-VI	20	8				1.40	
D-VII	20	1				0.57	
E-XI	20	5				4.14	
F-I	20	6				4.67	
G-III	20	10				1.73	
C-VI	25	6	6.0	0.75	0.84	1.24	1.87
E-XI	25	5		1.00		1.98	
F-I	25	5		0.83		3.35	
G-III	25	8		0.80		0.91	
A-II	30	10	4.0	0.91	0.55	1.47	1.15
D-VII	30	0		0.00*		1.97	
E-XI	30	1		0.20		0.95	
F-I	30	3		0.50		0.54	
G-III	30	6		0.60		0.80	
B-IV	35	9	9.0	0.82	0.82	1.16	1.16

*"D" series ratio excluded in average values

Table 10 presents summary data on the nitrification lag, t_N, value. As expected the value decreases with increasing temperature. Expressed in the form of a ratio, the value appeared to be quite consistent at the same temperature. The ratios also make it possible to use this information with greater applicability.

Another ratio is presented in the same Table which like the ultimate demand ratio noted previously, can be employed to characterize the effluent sample. This is the ratio of the nitrogenous velocity constant to the carbonaceous velocity constant. As noted previously the former value was greater on the average than the latter in this investigation. The secondary effluent used was apparently a well stabilized one from a carbonaceous view-point. It is also interesting to note that the ratio increased with decreasing temperature, indicating that the nitrogenous activity was more inherently reactive than the carbonaceous at lower temperatures.

One further item shown in Table 10 warrants comments. The t_N value at 20°C in some cases was less than or close to 5 days. This is significant because of the traditional practice of using the 5-day, 20°C BOD to characterize the strength of a waste water or effluent. An examination of the analyses in Appendix B will show that some amount of nitrification, usually nitrite formation, occurred in all of the 20°C test runs in 5 days. This situation should be of particular interest to plant operators when calculating removal percentages achieved in secondary plants, since these percentages are meant to reflect carbonaceous removals only.

VII. Relationship Between Deoxygenation Velocity Constant and Temperature

The primary purpose of this investigation was to arrive at some relationship between the rate of effluent deoxygenation and temperature. As stated earlier in the report, the usual relationship used by sanitary engineers is the Streeter-Phelps or the modified Arrhenius equation, which relates velocity constant to temperature. Other relationships have also been used, particularly in the biological literature, to express this dependence of reaction velocity on temperature. Examples of these will also be presented in the discussion that follows.

A. Arrhenius Relationship

The Arrhenius relationship as presented earlier in the report in equation (6) was written in a general form. For the purpose of this study, it would be more convenient to write the equation in terms of the 20°C conditions as follows:

$$\frac{k_T}{k_{20}} = \theta^{(T-20)} \quad \text{-----------------------} \quad (7)$$

where k_T and k_{20} are the values of the velocity constant at T°C and 20°C respectively and θ is, as before, the temperature coefficient. This is an exponential equation which means that a plot of the logarithm of either k_T or the ratio of k_T/k_{20} vs. temperature should yield a straight line for the range of temperatures in which θ is a constant. As noted previously, because of a 20°C control test run was included in each series, and the fact that the absolute value of k is of minor importance since it can vary from effluent to effluent, it was decided to calculate the θ values in terms of the ratios and compare the results with the θ values based also on the actual k values.

The θ values determined from the actual k values were arrived at by determining the line of best fit through the observed points by the method of "least square." In all cases the individual test run k values as well as the average values were plotted, though only the average values were employed in the evaluation of the θ value. When a break in θ value occurred, the second line of best fit was also established for the new temperature range, with the restriction however that it intersects the previous θ value at a common k value for the same temperature.

The θ values determined from the ratios were established in essentially the manner described above excepting that the line of best fit was forced through the value k_T/k_{20} equals one. This was done simply because the 20°C point represents a larger number of test runs than the other points and for this reason it was given greater emphasis, plus doing it this way eliminated the need for another constant in the resulting equations.

Another point should be noted regarding the relationships which are to be presented. The incubation temperatures used in the investigation range from 5° to 35°C. Only one test run each was conducted at the two temperature extremes. Major emphasis was directed to the range 10° to 30°C. The relationships presented will be confined to this range alone, however, it is of interest to note how well the observed points outside this range fit the established line.

1. Carbonaceous Deoxygenation

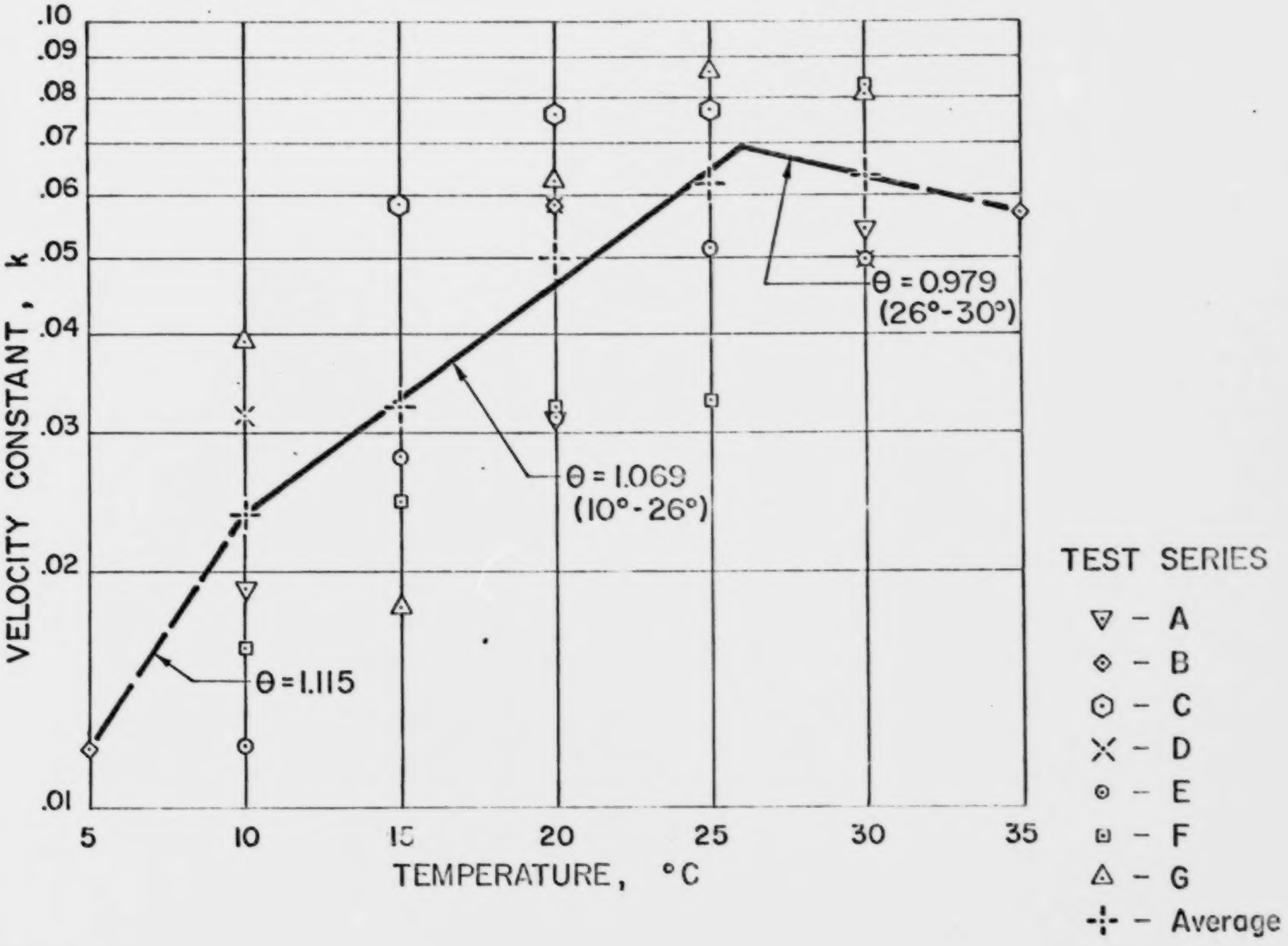
The relationship between the velocity constant, k, and the temperature, for the carbonaceous range, is presented in Figure 38. A break in the θ value was assumed to have occurred at 26°C. The equations of the two lines are as follows:

$$k_t = k_{20}(1.069)^{T-20} \qquad \text{Range } 10^\circ \text{ to } 26^\circ\text{C} - - - - (8)$$

$$k_t = k_{20}(1.493)(0.979)^{(T-26)} \qquad \text{Range } 26^\circ \text{ to } 30^\circ\text{C} - (9)$$

An additional constant must be included in the second equation because the line does not pass through the 20°C value of k and it has a negative slope.

67-a



Relationship Between Carbonaceous Velocity Constant and Temperature

FIGURE 38

Figure 39 presents the same relationship using the velocity constant ratio for the ordinate instead of velocity constant. It should be noted that the degree of variation in the observed points is significantly less in comparison with the previous plot. Selecting 20°C as the point for the break in θ values, the equation for the two lines are as follows:

$$\frac{k_t}{k_{20}} = (1.077)^{(T-20)} \quad \text{Range } 10° \text{ to } 20°\text{C} - - - - - (10)$$

$$\frac{k_t}{k_{20}} = (1.048)^{(T-20)} \quad \text{Range } 20° \text{ to } 30°\text{C} - - - - - (11)$$

2. Nitrogenous Deoxygenation

The relationship between velocity constant, k, and temperature for the nitrogenous range is presented in Figure 40. This plot shows that the optimum temperature for nitrification is at 20°C which is lower than that for the carbonaceous range. The equations for the two lines are as follows:

$$k_t = k_{20}(1.092)^{(T-20)} \quad \text{Range } 10° \text{ to } 20°\text{C} - (12)$$

$$k_t = k_{20}(0.123)(0.945)^{(T-20)} \quad \text{Range } 20° \text{ to } 30°\text{C} - (13)$$

Figure 41 presents the same relationship only in terms of the velocity constant ratio. In this case the break was selected at 22°C. The equation of the two lines are as follows:

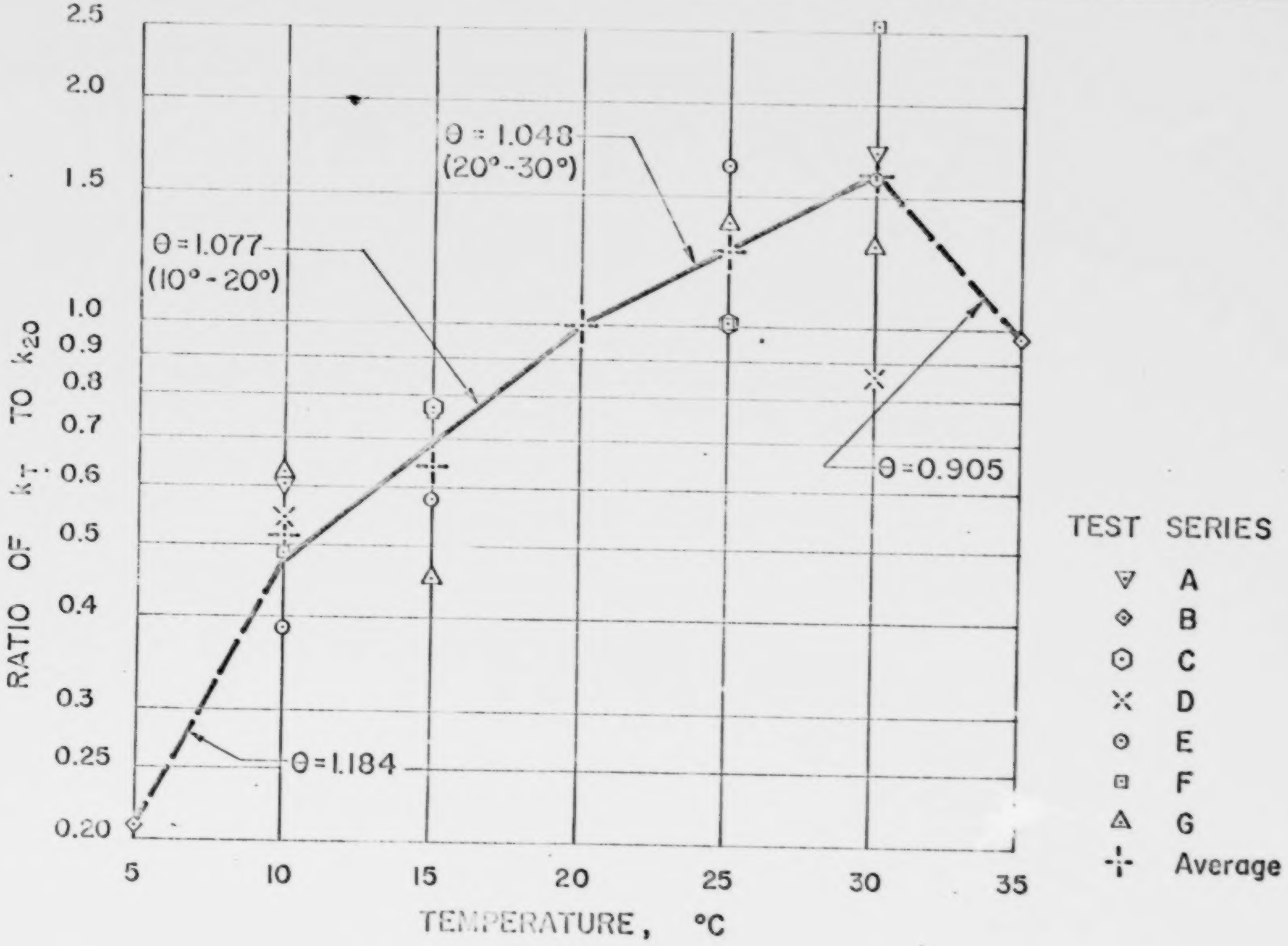


FIGURE 39

Relationship Between Carbonaceous Velocity Constant Ratio and Temperature

68-b

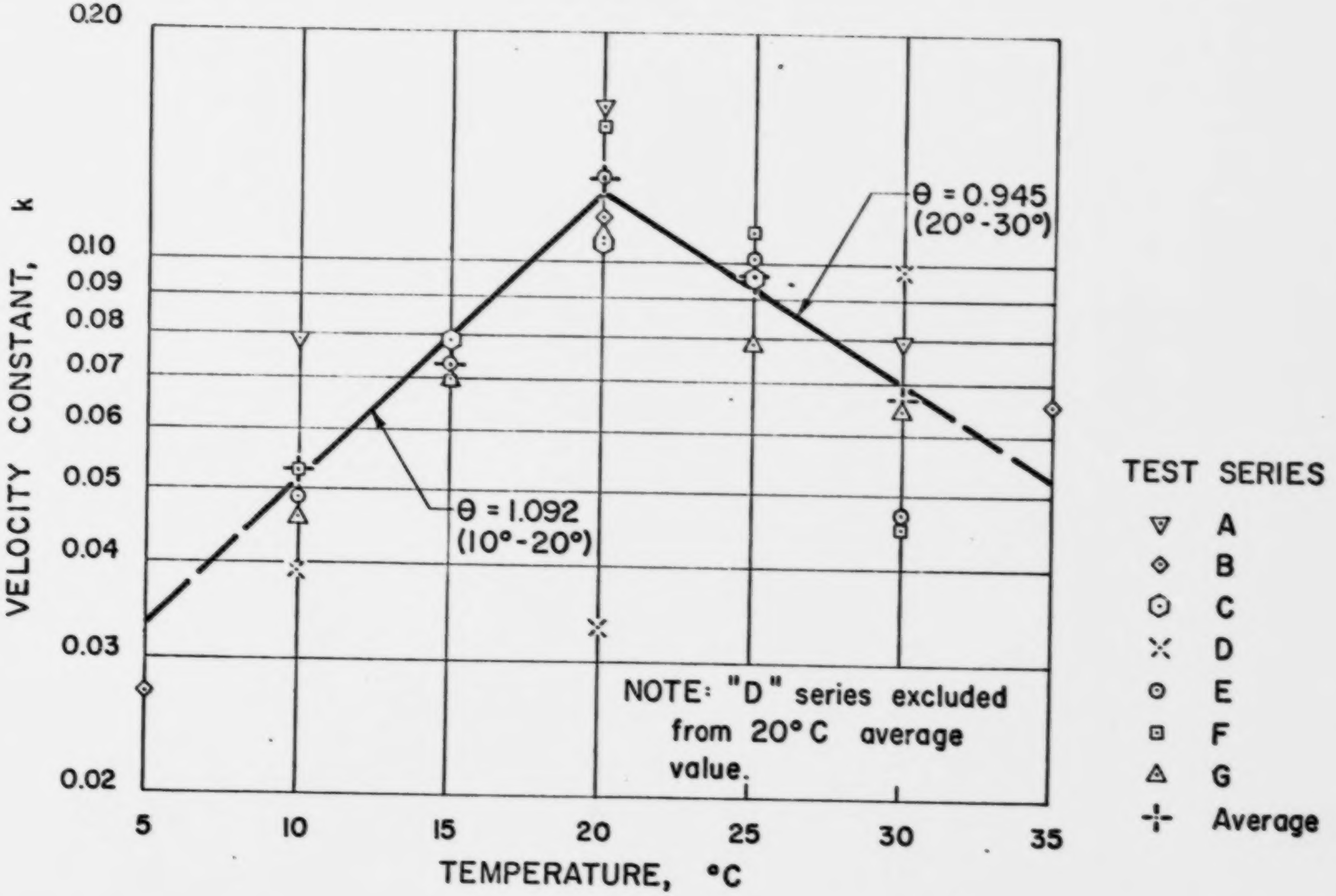


FIGURE 40

Relationship Between Nitrogenous Velocity Constant and Temperature

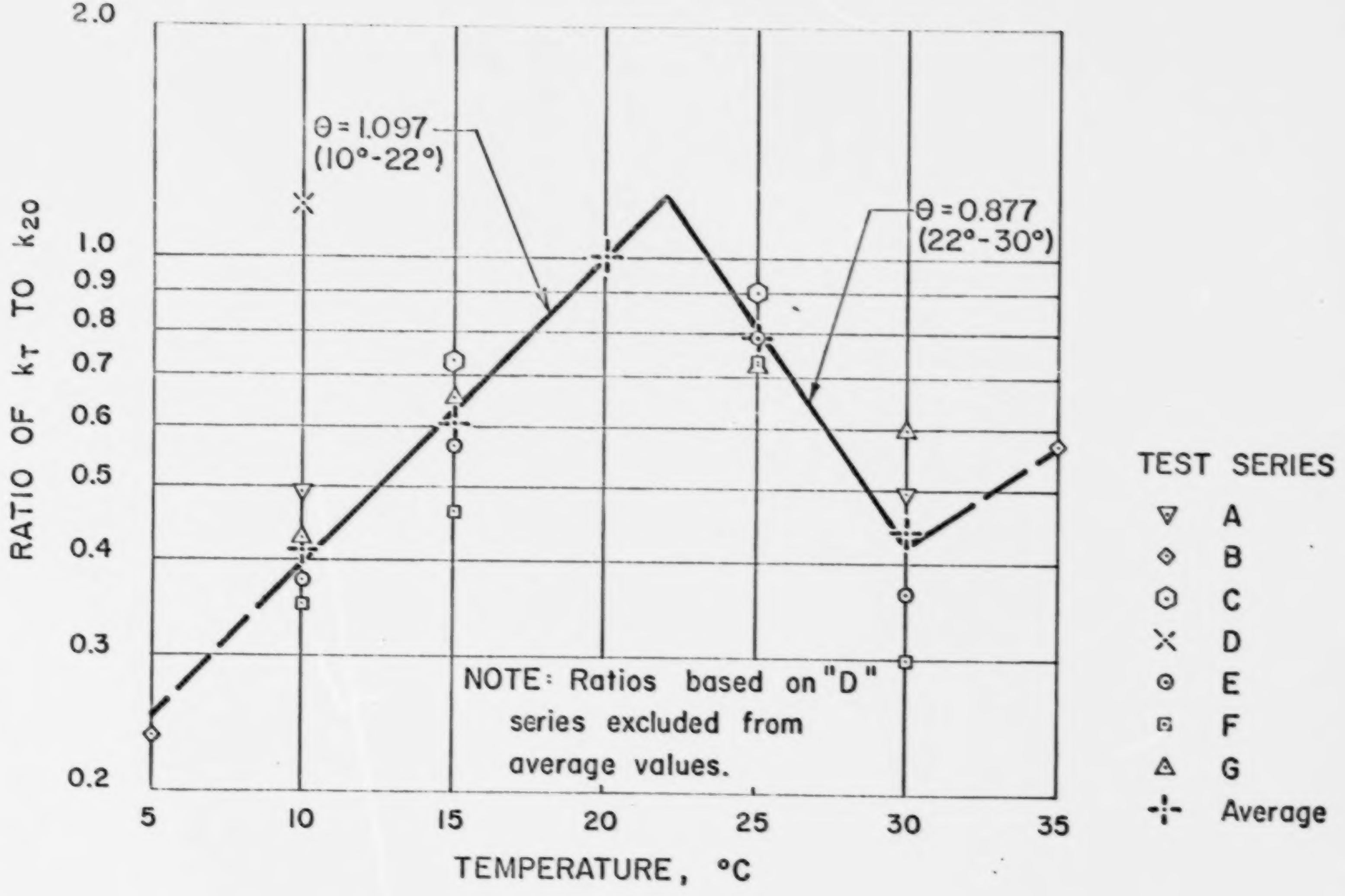


FIGURE 41

Ratio Between Nitrogenous Velocity Constant Ratio and Temperature

68-c

$$\frac{k_t}{k_{20}} = 1.097^{(T-20)} \quad \text{Range } 10° \text{ to } 22°\text{C} \text{ - - - - - } (14)$$

$$\frac{k_t}{k_{20}} = (1.203)(0.877)^{(T-22)} \quad \text{Range } 22° \text{ to } 30°\text{C} \text{ - - } (15)$$

3. Activated Energy Value

Often in the biological literature a term referred to as the "activation energy" or "temperature characteristic" is derived from reaction kinetics data at varying temperatures. This value (μ) can be calculated from the original Arrhenius equation as follows:

$$\log \frac{k_1}{k_2} = \frac{\mu(T_1' - T_2')}{2.3026R(T_1')(T_2')} \quad \text{- - - - - - - - - - } (16)$$

where k_1 and k_2 are the values of the velocity constant as the absolute temperatures T_1' and T_2', and R is the universal gas constant which has a value of 1.99 calories. The value of μ is actually related to the slope of the velocity constant--temperature plot, or in other words, the θ value. It has been found that certain types of biological reactions will have a certain narrow range of activation energy values for a constant temperature range. In fact these values are often used in an attempt to characterize the type of reaction (oxidation, hydrolysis, etc.) which is governing the overall observed activity. The μ values presented below were calculated from the plots based on velocity constant ratios.

Temperature Range	Deoxygenation Type	μ
10° to 20°C	Carbonaceous	12,270
20° to 30°C	Carbonaceous	8,380
5° to 22°C	Nitrogenous	15,100
22° to 30°C	Nitrogenous	-23,350

B. Other Relationships

Two other relationships, among others, have been used in the biological literature to characterize the dependence of the reaction rate on temperature. They are Belehradek's empirical expression, and the van't Hoff or Q_{10} value. An attempt was made to describe some of the results of this study by these methods.

1. Belehradek's Empirical Expression

In 1930, Belehradek[32] wrote a very comprehensive paper, somewhat critical of the expressions used at that time, for relating temperature to reaction rate. He suggested the following empirical formula which he found to fit the observed data with a high degree of accuracy in many cases:

$$y = \frac{a}{T^b} \qquad (17)$$

where, T = temperature in °C

y = time required to accomplish a reaction

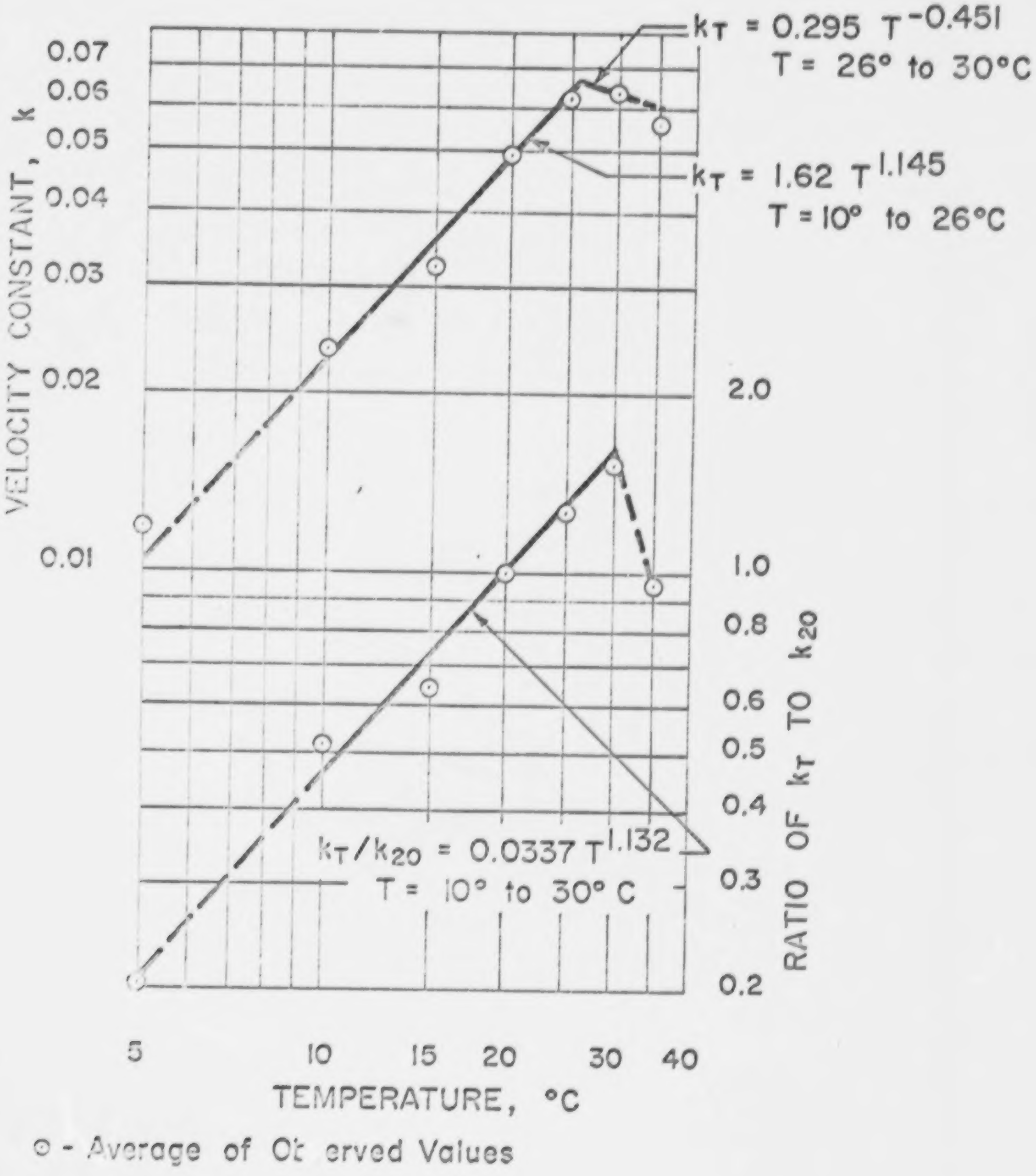
He felt that this expression had more logical basis as the temperature was measured from "biological zero" or 0°C, instead of from absolute zero as is used in the Arrhenius expression. Belehradek further observed that for many biological reactions the value of "b" is often equal to unity.

The carbonaceous deoxygenation velocity constants were plotted vs. temperature on a log-log paper as shown in Figure 42. The reasonably good fit to a straight line indicates that the data can be described by this empirical equation employing either the absolute values or the ratios. Even the 5°C results seemed to be in accordance with this expression. The equations for the lines are shown in the figure. Interestingly, the ascending portion of both plots resulted in a temperature exponent "b" of approximately 1.1.

2. The van't Hoff or Q_{10} Value

In 1884, van't Hoff generalized that when the ratio of velocity constants is determined for a range of 10 degrees centigrade, the resulting figure should be in a vicinity of 2 to 3. This has led to the general rule of thumb often mentioned that the velocity of biological reaction doubles for each 10 degree rise in temperature. The ratio is often referred to as the Q_{10} value. Though this parameter is admittedly an approximate one, primarily because it fluctuates greatly with the temperature range considered, it is still frequently used in the biological literature as an approximate guide. The Q_{10} values were determined for the average carbonaceous and nitrogenous velocity constants as follows:

$Q_{10} = \frac{k_{(T+10)}}{k_T}$ and are presented below:



Belehradek's Empirical Expression Relating Carbonaceous Velocity Constant and Temperature

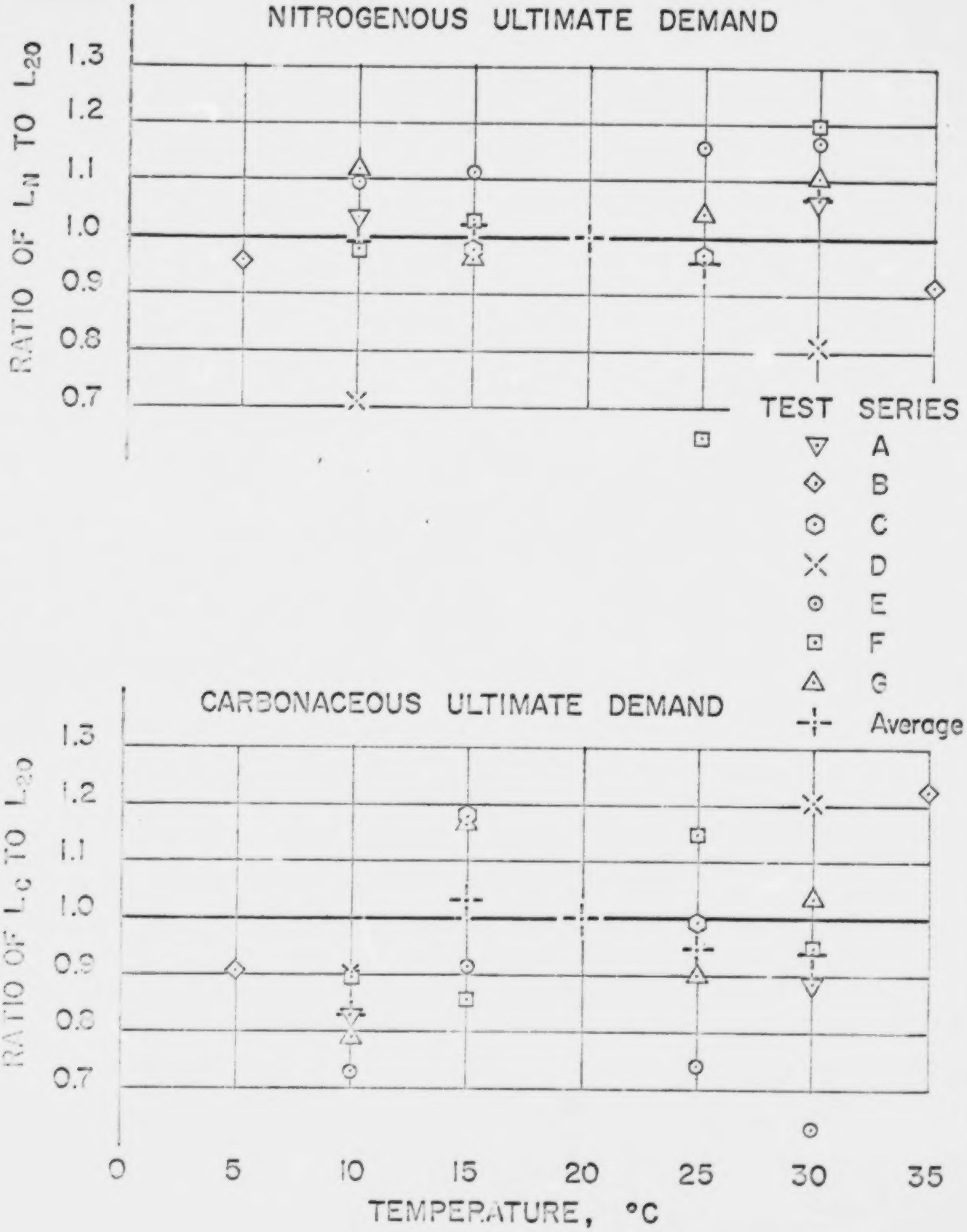
FIGURE 42

Temperature Range °C	Q_{10} Values	
	Carbonaceous	Nitrogenous
5-15	2.71	2.69
10-20	2.12	2.42
15-25	1.92	1.32
20-30	1.27	0.52
25-35	0.92	0.68

The above results demonstrate that the Q_{10} value is a poor parameter for characterizing the effect of temperature on reaction rate. The results are in the usual biological range and decrease in value with increasing temperature range as is usually the case with biological reactions.

VIII. Relationship Between Ultimate Demand and Temperature

The ultimate demand values presented previously in Table 9 indicated that they did not vary significantly with temperature, within the same test series. This situation was found to be true for both the carbonaceous and the nitrogenous ultimate demands. This is significant since all the test runs of the same series were set up with the same effluent sample. Figure 43 presents a plot of the ultimate demand in the form of ratios plotted against temperature for both deoxygenation stages. As noticed the values cluster above and below the 1.0 value with no discernable upward and downward trend. It is safe to conclude from these results that for secondary effluents neither the carbonaceous nor the nitrogenous ultimate demands vary significantly with temperatures.



Relationship Between Ultimate Demand and Temperature FIGURE 43

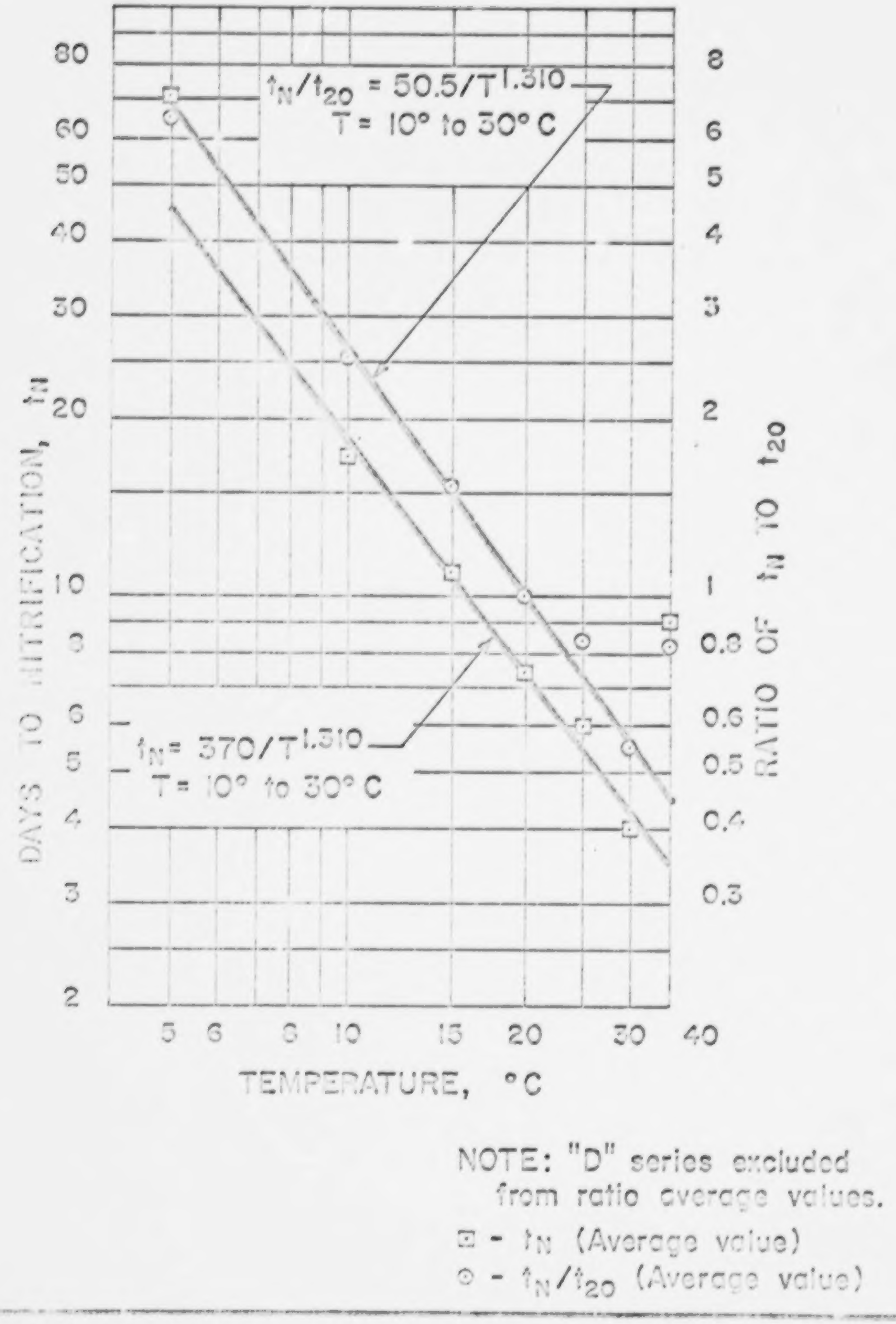
IX. Relationship Between Nitrification Lag and Temperature

Any practical application of the deoxygenation data would require some knowledge of the relationship between the extent of nitrification lag and temperature. After investigating various different possibilities, it was found that this relationship can best be described by a plot on log-log paper as shown in Figure 44. Both the absolute t_N value in days and the ratios can be described quite well in this manner, at least within the range of 10 to 30°C. The equation for both lines is shown directly on the plot. Figure 45 and 46 represent the two calculated curves plotted on rectangular coordinate paper with the results of all the test series.

X. Comparison With the Results of Other Investigations

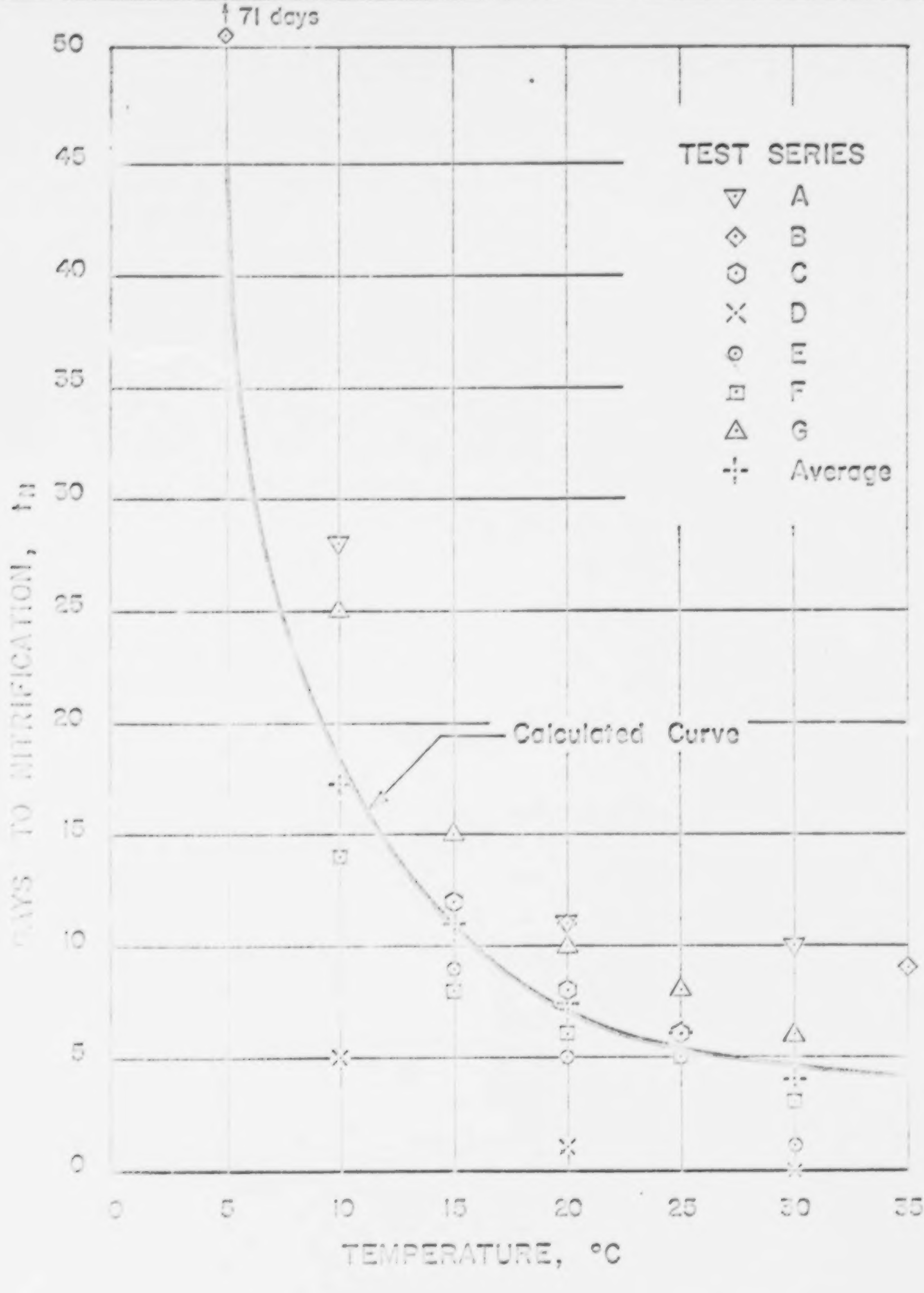
The relationship between temperature and both the carbonaceous and nitrogenous velocity constants, developed in this study, for the secondary effluents, is shown in Figure 47 in conjunction with the results of other investigations reported in the literature. A comparison of this type was presented by the writer[15] before, the only difference being that the results of this study are included. The results of this study which are shown in the figure are taken from the previously presented Figures 39 and 41.

To realistically compare all the results shown in the figure, consideration must be given to the type of the substrate, method of evaluating constants, and analytical procedures employed in each case.



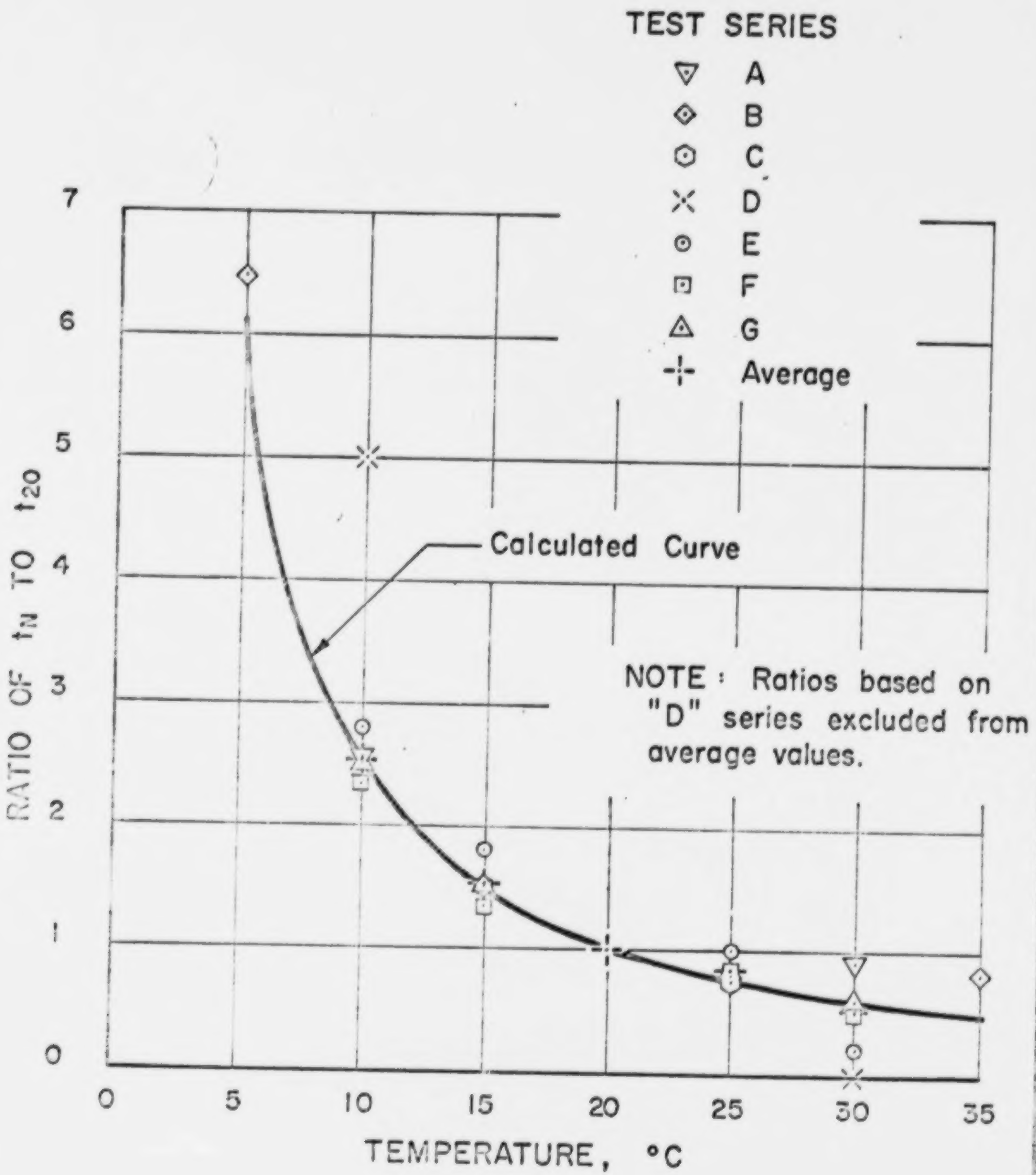
Empirical Relationship Between Nitrification Lag and Temperature

FIGURE 44



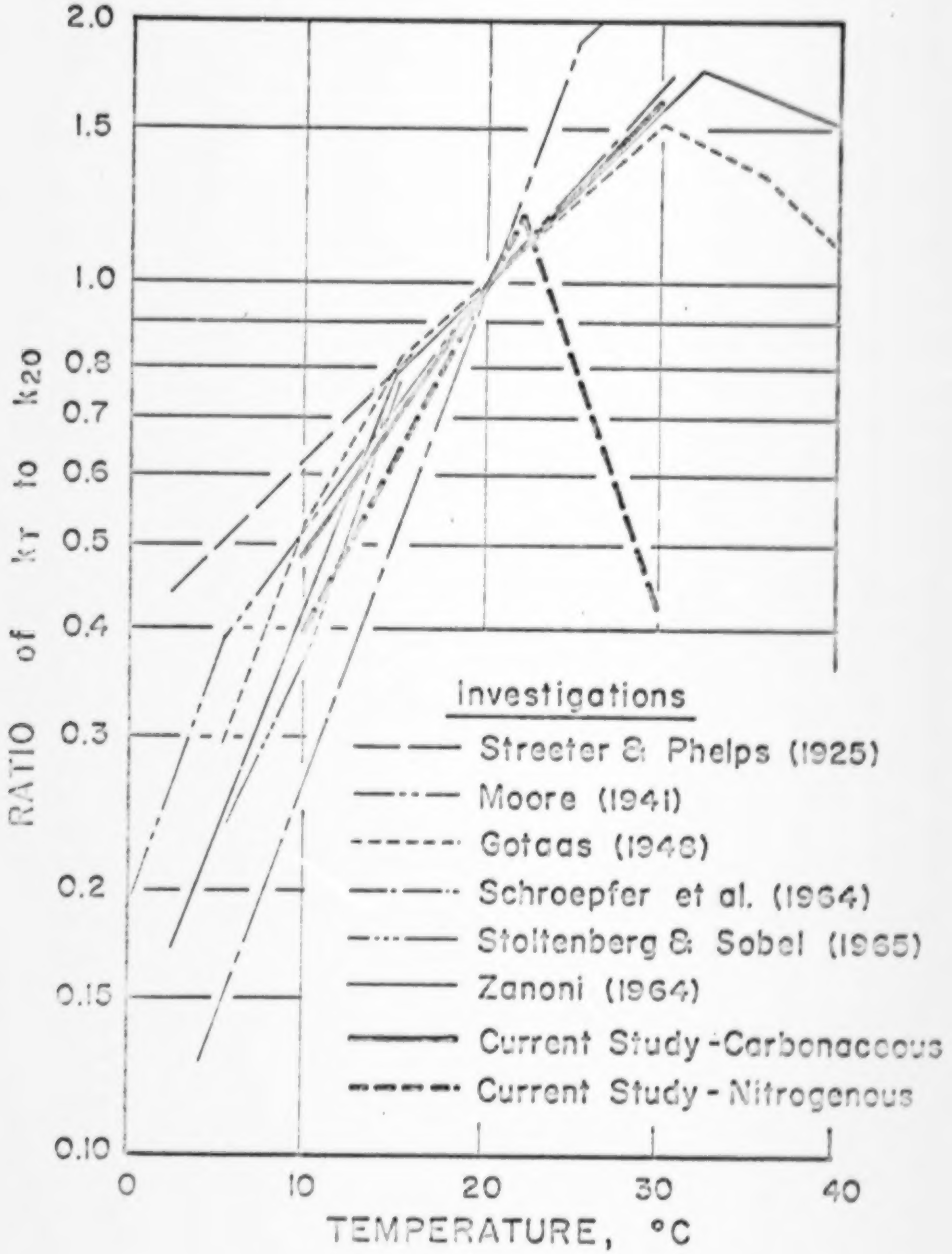
Relationship Between Nitrification Lag and Temperature Showing Calculated Curve

FIGURE 45



Relationship Between Nitrification Lag Ratio and Temperature
Showing Calculated Curve

FIGURE 46



Velocity Constant Comparison with Past Investigations

FIGURE 47

A summary of this type of information was presented in Table 1 earlier in the report.

As noted, the carbonaceous relationship fits in very well with the results of other investigators regardless of the type of substrate involved. This is particularly true of the 20 to 30°C temperature range where the θ obtained from this study was 1.048. This value checks surprisingly well with the traditional value of 1.047 originally established by Streeter and Phelps[1] back in 1925. The 10 to 20°C results are also in line with those of the past, representing almost an average value of all the past results shown. Even the extension of the results of this study to 5°C and 35°C fit in well, but these were not shown since only one test run was conducted at each of these temperatures.

The nitrogenous deoxygenation results, however, are strikingly different from those reported in the past. It must be remembered that all the past studies shown are for the carbonaceous range and to the best of the writer's knowledge none of this type have ever been reported for the nitrogenous activity. The major difference in the nitrogenous relationship is in the higher temperature range. From 10 to 22°C the behavior is not markedly different from that exhibited by the carbonaceous deoxygenation. The optimum or maximum rate occurs at 22°C and from this temperature to 30°C the velocity constant decreases. This decrease in activity rate for temperatures above 22°C cannot be refuted if one examines the results of the individual test series at the 25°C and 30°C incubation temperatures.

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It would seem difficult to defend the 22°C optimum value obtained in this investigation in view of the fact that the optimum growth temperature for nitrifiers is often reported as high as 30°C.[3,33,34] However, the optimum temperature for the growth of nitrifiers in pure culture need not necessarily correspond with the optimum temperature for the nitrogenous deoxygenation of secondary effluents measured in terms of oxygen usage. An examination of the previously presented figures, with the individual test run results, will show that at temperatures of 20°C and below there is more of an abrupt change from the first to the second stage. The longer nitrification lag at these temperatures allow for the development of a well acclimated nitrifier population which appears to nitrify almost precipitously after achieving a certain minimal population level. The nitrate-formation activity is concurrent with, or follows immediately after, the nitrite-formation activity. The combination of all these factors tends to increase the rate of nitrification once it has commenced, even though the nitrifier population changes might result in a somewhat different pattern. At incubation temperatures above 20°C there was more of a transitional change from one stage to the other. Nitrite formation commenced almost immediately followed by the nitrate formation in a somewhat distinct second step. This "spreading out" of the two nitrification steps had the effect of lowering the velocity constant.

The fact that the nitrification velocity constant at say 20°C is higher than that at 30°C does not mean that the total oxygen demand exerted by a secondary effluent after a certain period of time is necessarily greater at the lower temperature. The values of the carbonaceous velocity constant and the nitrification lag must still be taken into account. If one, for example, assumes a situation where the ultimate demand value for both stages was equal to 100 mg/l each, and k, θ and t_N values are derived from this investigation, after 10 days 69 percent of total ultimate demands will be exerted at 30°C, whereas at 20°C, this value is 63 percent.

It is also of interest to compare the relationship between temperature and the first and second stage ultimate demands of this study with what has been reported in the past. Regarding the carbonaceous demand, Theriault,[2] Moore,[10] and Zanoni[15,16] all concluded that this ultimate demand did vary somewhat with temperature. Gotaas,[11] on the other hand, concluded that for all practical purposes it did not vary. Again considerations must be given to test substrate and laboratory procedures in each case to make a valid comparison. In the case of raw waste waters, with substantially greater ultimate demands, there is a greater likelihood of obtaining more definitive experimental evidence of such a relationship if one actually exists. The much lower ultimate demand of the secondary effluents makes it more difficult to delineate any positive upward or downward trend, since it is known that the degree of variation, if it does exist, would be quite small. It is interesting that if the sum of the carbonaceous and nitrogenous ultimate demands for the lowest and highest temperatures of each test series is compared, in most cases the sum is greater at the higher temperatures. However, from the results of this experiment, at least for the 10 to 30°C temperature range, it must be concluded that neither the carbonaceous nor nitrogenous ultimate demand of a secondary effluent, for all practical purposes, varies significantly with temperature.

XI. Summary

This study was concerned with the long term carbonaceous and nitrogenous deoxygenation of conventional activated sludge treatment process effluent at incubation temperatures ranging from 5 to 35°C. Biochemical oxygen demand determinations were conducted using a "jug" dilution technique at numerous time periods along the deoxygenation progression. Because of the nature of the test substrate, special consideration was given to the nitrogenous phase of the deoxygenation by conducting numerous nitrite and nitrate nitrogen analyses in addition to the oxygen demand measurements. After completing four test series including three test runs in each, the decision was made to confine the investigation to the 10-30°C temperature range. A minimum of four repetitive test runs were eventually set up at each incubation temperature employed.

The first step in the analysis of the laboratory data was the evaluation of the first and second stage monomolecular deoxygenation constants k and L using the so-called "moments" method. The nitrogen analyses were used in conjunction with the BOD values to separate the carbonaceous stage from the nitrogenous stage to facilitate the calculations.

With the deoxygenation constants evaluated for all the individual test runs, an attempt was made to relate these data to the incubation temperature. The traditional Arrhenius expression was employed to relate velocity constant to temperature. This relationship is characterized by the θ value or the temperature coefficient. For the carbonaceous phase of deoxygenation the best value of θ was found to be 1.077 for the temperature range of 10 to 20°C, and 1.048 for the temperature range of 20 to 30°C. These results compare closely with those of past investigations in which raw waste waters and river water were used as test substrates. For the nitrogenous phase the values of 1.097 and 0.877 were obtained for the temperature ranges of 10 to 22°C, and 22 to 30°C respectively. These results are interesting in comparison to the carbonaceous deoxygenation data because of the relatively low optimum temperature of 22°C which resulted.

An attempt was also made to use other expressions from the biological literature to characterize the relationship between the velocity constant and the temperature. These results were found to be consistent with what one would expect for biochemical type reactions.

For all practical purposes both the calculated carbonaceous and nitrogenous ultimate demands did not vary significantly with temperature. The results of the past investigations do not agree in this respect though no data is available for secondary effluents.

Finally, an empirical expression was derived relating temperature to nitrification lag or the time in days from the start of the test to the onset of active nitrification.

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TABLE A-1

Actual NO_3-N Concentrations Determined by the Phenoldisulfonic Acid Method with Varying Chloride Concentrations Showing Per Cent Error Due to Chloride Interference

		Actual NO_3-N in mg/l						
	0	0.10	0.20	0.30	0.40	0.50	0.6	
	5	0.080 20.0	0.165 17.5	0.256 16.7	0.323 19.2	0.430 14.0	0.528 12.0	mg/l (%)
Cl^- Added in mg/l	10	0.070 30.0	0.155 22.5	0.227 24.3	0.285 28.7	0.390 22.0	0.465 22.5	
	50	0.065 35.0	0.140 30.0	0.212 29.3	0.280 30.0	0.334 33.2	0.352 41.3	
	100	0.060 40.0	0.122 39.0	0.192 36.0	0.230 42.5	0.275 45.0	0.352 41.3	
	150	0.062 38.0	0.115 42.5	0.195 35.0	0.212 47.0	0.235 53.0	0.380 36.6	
	200	0.060 40.0	0.115 42.5	0.180 40.0	0.212 47.0	0.270 46.0	0.340 43.3	
	300	0.038 62.0	0.115 42.5	0.177 41.0	0.210 47.5	0.210 58.0	0.225 45.8	
	400	0.020 80.0	0.070 65.0	0.175 41.6	0.212 47.0	0.202 59.6	0.205 49.1	

TABLE B-1

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN A-II-10 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.040	0.460
4	7.0		
7	10.3	0.035	0.440
12	14.2		
14	17.0	0.088	0.0
17	17.6		
21	20.6	0.285	0.340
25	24.3		
28	29.9	1.100	0.785*
30	33.7		
32	39.7		
35	51.2	0.050	6.300
37	55.8		
40	56.3		
42	56.9		7.000
46	59.2		6.950
56	60.2		
63	61.1		7.000
70	61.8		
77	63.9		
83	66.5		
91	65.1		

*Questionable accuracy because of high nitrite value

TABLE B-2

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN A-II-20 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.067	0.450
4	10.3		
5	10.9		
7	14.9	0.152	.460
12	32.4		
14	40.8	1.420	1.365*
17	57.0		
18	60.8		
19	61.8		
21	63.0	0.055	6.825
23	63.1		
25	65.0		
28	66.3	0.050	7.000
32	66.7		
35	67.5	0.020	7.075
39	68.2		
42	69.1	0.0	6.825
46	69.4		7.000
49	70.7		

*Questionable accuracy because of high nitrite value

TABLE B-3

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN A-II-30 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	—	0.035	0.460
2	8.5		
3	12.5		
4	15.3		
5	18.6		
7	24.3	0.230	0.440
11	34.3		
12	37.1		
13	43.2		
14	48.7	5.620	0.655*
17	55.8		
18	58.7	0.0	
19	59.7		
21	61.3	0.0	6.950
23	61.9		
25	62.7		
28	63.5	0.0	7.725
32	66.1		
35	66.7	0.0	7.075
39	68.1		
42	68.4	0.0	7.000
46	69.5		7.000
49	70.3		

*Questionable accuracy because of high nitrite value

TABLE B-4

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN B-IV-5 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.048	0.33
5	5.4		
7	8.2	0.043	0.30
10	11.0		
14	13.3	0.048	0.26
17	15.0		
21	18.2	0.048	0.56
24	18.6		
27	22.2	0.060	0.26
31	21.7	0.055	
35	24.6	0.095	0.40
39	23.3	0.110	
41	22.9		
45	27.4	0.147	
49	29.0	0.225	1.04
52	29.6	0.269	
56	31.0	0.259	1.06
59	31.9	0.145	
63	32.6	0.067	1.94
70	36.4		3.86
77	39.3		
84	40.9		
41	49.1	0.012	
98	52.5		4.13
105	54.4	0.005	4.42
112	55.7		4.58
119	58.5		
126	56.9		
132	55.9		
147	57.9		
154	57.6		4.13

TABLE B-5

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN B-IV-20 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.052	0.455
1	7.2	0.034	
2	12.1	0.036	
3	14.9	0.048	
4	17.2	0.053	
5	20.1	0.067	
6	22.1	0.088	
7	25.0	0.205	0.225
8	29.0	0.305	
9	29.2	0.491	
10	31.6	0.899	
11	34.4	1.463	
12	37.9	2.156	
13	44.6	2.438	
14	47.2	1.475	2.065*
15	53.2	0.319	
16	53.8	0.030	
17	55.7	0.0	
18	56.4	0.0	
19	56.9		
21	57.7	0.035	3.685
24	59.4		
27	61.0	0.010	4.980
31	62.3		
35	62.9		5.020
39	64.6		
41	64.4		

*Questionable accuracy because of high nitrite value

TABLE B-6

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN B-IV-35 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.065	0.350
1	9.4	0.055	
2	16.0	0.045	
3	19.6	0.065	
4	21.8	0.055	
5	25.1	0.080	
6	28.2	0.090	
7	29.3	0.203	0.725
8	32.4	0.305	
9	34.6	0.699	
10	39.3	1.724	
11	47.9	3.975	
12	50.5	3.975	
13	51.8	4.000	
14	53.3	4.025	0.225*
15	54.5	3.794	
16	55.8	3.475	
17	56.5	2.200	
18	60.5	0.167	
19	64.6	0.0	
21	62.5	0.02	3.980
24	66.2		
27	72.3		4.660
31	69.6		
35	72.2		4.560
39	68.9		

*Questionable accuracy because of high nitrite value

TABLE B-7

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN C-IV-15 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	—	0.044	0.0
1	7.9	0.057	
2	11.7	0.053	
3	14.3	0.063	
4	17.8	0.070	
5	19.8	0.080	0.470
6	21.6	0.137	
7	24.4	0.162	0.510
8	26.0	0.273	
9	28.5	0.315	
10	29.5	0.470	
11	33.0	0.708	0.590
12	34.8	1.150	
13	37.9	1.625	
14	41.9	2.125	1.130*
15	44.6	2.325	
16	49.4	2.054	
17	55.7	1.450	
18	60.3	0.631	5.72
19	62.9	0.142	6.58
20	64.3	0.004	
21	65.5	0.012	
23	66.8		
25	67.8		
28	71.0		7.600
32	72.5		
35	73.4		7.600
37	73.2		7.700

*Questionable accuracy because of high nitrite value

TABLE B-8

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN C-VI-20 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.040	0
1	8.3	0.045	
2	11.7	0.060	
3	15.0	0.088	
4	18.7	0.133	
5	22.9	0.215	0.480
6	24.0	0.440	
7	27.9	0.738	0.470
8	30.8	1.676	
9	34.6	2.505	
10	39.4	3.761	2.019*
11	46.2	4.319	3.131*
12	52.0	3.619	4.606*
13	57.0	1.563	6.000
14	59.7	0.532	7.350
15	61.0	0.068	7.775
16	61.7	0.023	
17	62.1	0.010	
18	62.6		7.225
22	64.9		
25	66.5		
28	67.8		8.200
32	69.1		
35	70.6		8.200
37	71.0		8.125

*Hydrogen peroxide oxidation employed because of high nitrite values but analyses still of questionable accuracy

TABLE B-9

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN C-VI-25 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.0325	0.0
1	6.9	0.0570	
2	10.6	0.090	
3	14.0	0.1575	
4	17.6	0.290	
5	21.9	0.610	0.475
6	25.9	1.380	
7	31.4	2.328	0.390*
8	36.4	4.058	0.642*
9	42.2	4.607	
10	48.4	4.444	3.856*
11	51.9	3.738	4.412*
12	55.7	2.791	4.830*
13	57.2	1.600	5.625
14	59.2	0.318	7.250
15	60.6	0.014	7.625
16	60.7	0.033	
17	62.2	0.016	
18	62.5	0.010	6.975
22	63.9		
25	64.8		
28	65.6		7.625
32	68.8		
35	69.4	0.0	8.150
37	69.7	0.0	8.150

*Hydrogen peroxide oxidation employed because of high nitrite values but analyses still of questionable accuracy

TABLE B-10

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN D-VII-10 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.071	0.33
1	0.4	0.082	
2	2.3		
3	3.9	0.119	
5	7.9	0.095	
6	10.4	0.106	
8	14.2	0.075	
10	15.2	0.064	1.18
12	19.2	0.056	1.20
14	20.7	0.027	2.01
16	22.1	0.040	2.16
17	22.5	0.023	2.25
20	25.1	0.004	2.63
22	24.5	0.0	
24	26.2		3.07
28	27.7		3.09
31	30.7		3.07
35	29.6		
38	32.1		
44	29.9		
59	33.9		3.40
66	34.5		3.07
80	34.8		
87	35.6		

TABLE B-11

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN D-VII-20 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	—	0.064	0.28
1	4.1	0.131	
2	7.3	0.234	
3	10.5	0.317	
4	13.8	0.835	
5	18.9	0.370	
6	19.7	0.335	1.33
7	21.5	0.282	1.56
8	24.1	0.188	1.71
9	24.7	0.121	1.81
10	27.0	0.040	2.76
11	27.8	0.015	2.60
12	29.2	0.004	2.63
13	29.8	0	2.70
14	31.0	0	2.70
15	31.6	0	2.81
16	32.3		2.81
17	32.2		2.77
20	33.4		
24	35.7		3.12
28	36.2		
31	36.4		3.17
35	38.3		
38	38.6		
42	39.6		
44	40.0		
54	41.4		4.25
59	42.1		4.50
66	43.4		3.40

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TABLE B-12

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN D-VII-30 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.075	0.19
1	6.7	0.284	
2	14.4	0.554	
3	17.5	0.676	
4	21.2	0.898	2.67
5	23.2	0.910	1.84
6	25.5	1.030	1.68*
7	27.0	0.940	2.41
8	28.4	0.843	1.91
9	30.2	0.711	2.11
10	30.4	0.525	2.26
11	31.2	0.317	2.41
12	32.2	0.174	2.41
13	34.0	0.079	2.76
14	34.2	0.048	
15	35.0	0	2.81
16	36.3	0	3.07
17	36.5	0.004	3.07
20	39.4		3.43
24	40.6		
28	42.6		
31	42.1		3.17
35	42.9		
38	44.9		3.80

*Questionable accuracy because of high nitrite value

TABLE B-13

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN E-XI-10 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.097	0.15
1	1.0	0.100	
2	2.0	0.108	
3	3.2	0.135	
4	3.4	0.145	
5	4.4	0.200	
6	4.6	0.222	
7	6.0	0.252	0.30
9	6.6	0.295	
11	8.6	0.385	
13	10.5	0.520	
14	11.4	0.650	0.62
16	14.2	0.805	
19	20.5	0.820	
21	25.3	0.615	4.00
24	31.6	0.110	
28	38.2	0.035	6.60
31	39.3	0.031	
35	41.0	0.028	6.30
38	41.5	0.045	
41	42.5		
42		0.060	5.80
45	43.7	0.047	
49	45.0	0.035	6.60
52	45.3	0.031	
56	46.7	0.035	6.50
63	48.7	0.035	6.60
70	48.5	0.040	6.80
77	49.4	0.040	6.30
91	50.7	--	7.04

TABLE B-14

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN E-XI-15 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.090	0.15
1	2.0	0.105	
2	3.1	0.132	
3	5.1	0.180	
4	6.6	0.237	
5	7.2	0.307	
6	9.5	0.387	
7	10.8	0.537	0.35
8	12.2	0.695	
9	14.3	0.925	
10	15.6	1.200	
11	21.6	1.312	
12	23.4	1.625	
13	25.5	1.562	
14	26.9	1.000	3.50
16	35.9	0.150	
19	41.8	0.035	
21	43.4	0.030	3.60
24	45.2	0.040	
28	46.80	0.045	6.90
31	48.60	0.030	
35	50.0	0.035	6.30
38	50.6	0.035	
41	51.8		
42		0.072	6.20
45	52.9	0.047	
49	53.5	0.035	6.90
52	54.1	0.030	
56	55.0	0.030	6.80
63	55.8	0.028	7.10
70	57.0	0.022	6.90
77	58.0	0.030	6.10
91	58.9	--	7.40

TABLE B-15

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN E-XI-20 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.092	0.15
1	3.3	0.137	
2	4.8	0.207	
3	7.5	0.340	
4	9.5	0.512	
5	11.3	0.845	
6	15.3	1.300	
7	20.7	2.075	1.50*
8	25.9	2.475	
9	33.6	2.500	
10	37.4	1.725	
11	39.4	0.650	
12	42.9	0.117	
13	44.7	0.040	
14	46.5	0.036	5.50
16	47.9	0.036	
19	50.3	0.036	
21	50.3	0.030	6.80
24	51.7	0.027	
28	52.9	0.082	6.50
31	53.7	0.036	
35	54.3	0.030	4.84
38	54.8	0.030	
42	55.8	0.025	5.10
45	56.6	0.036	
49	56.6	0.036	5.36
52	57.7	0.030	
56	58.3	0.030	5.24

*Questionable accuracy because of high nitrite value

TABLE B-16

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN E-XI-25 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--		
1	2.7	0.155	0.15
2	6.2	0.252	
3	8.5	0.430	
4	10.5	0.780	
5	13.8	1.400	
6	21.5	1.950	
7	27.9	2.375	1.50*
8	31.3	2.100	
9	35.4	1.150	
10	38.9	0.212	
11	41.1	0.047	
12	41.5	0.070	
13	44.8	0.031	
14	43.1	0.027	5.00
16	43.4	0.055	
19	46.4	0.031	
21	48.1	0.055	6.30
24	50.0	0.075	
28	52.7	0.031	7.50
31	53.6	0.025	
35	54.0	0.027	7.20
38	54.1		
42	54.7	0.025	7.20
45	54.9	0.031	
49	56.3	0.027	7.40

*Questionable accuracy becuase of high nitrite value

TABLE B-17

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN E-XI-30 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.072	0.15
1	3.7	0.170	
2	7.9	0.295	
3	12.7	0.586	
4	15.7	1.100	
5	21.8	1.925	
6	26.4	2.675	
7	26.8	3.125	1.90*
8	28.7	2.950	
9	30.5	2.100	
10	32.6	1.100	
11	35.1	0.325	
12	37.0	0.060	
13	36.6	0.027	
14	37.15	0.027	5.50
16	37.7	0.055	
19	41.4	0.027	
21	43.8	0.024	6.20
24	45.7	0.050	
28	47.6	0.015	6.80
31	48.1	0.040	
35	48.6	0.027	6.80
38	51.9		
42	51.4	0.024	6.90
45	52.0	0.031	
49	52.1	0.031	7.24

*Questionable accuracy because of high nitrite value

TABLE B-18

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN F-I-10 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.105	0.15
1	1.4	0.115	
2	3.0	0.125	0.27
3	4.4	0.127	
4	5.1	0.150	
5	7.1	0.165	
6	7.6	0.182	0.30
7	8.2	0.212	
8	9.2	0.242	
9	8.5	0.300	0.35
10	10.0	0.375	
11	12.4	0.425	
12	14.3	0.505	
13	15.3	0.625	
14	17.1	0.887	
15	17.5	1.075	
16	19.2	1.237	0.57*
17	20.8	1.450	
20	25.20	2.025	
21		1.987	
23	31.9	1.137	2.50
27	39.1	0.068	
30	40.8	0.032	3.70
34	48.9	0.032	
37	44.9		5.00
41	46.4		
44	46.7		4.60
50	47.9		4.50
58	48.9		5.00
65	49.6		5.90

*Questionable accuracy because of high nitrite value

TABLE B-19

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN F-I-15 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.105	0.15
1	2.3	0.125	
2	4.2	0.147	0.27
3	6.0	0.170	
4	7.4	0.222	
5	9.7	0.280	
6	11.0	0.365	0.42
7	11.6	0.485	
8	13.3	0.687	
9	15.7	0.887	0.42
10	18.9	1.187	
11	22.0	1.537	
12	25.1	1.625	
13	27.1	1.887	
14	30.4	1.937	
15	32.9	1.400	
16	35.5	0.710	3.75
17	37.8	0.262	
20	42.1	0.050	
23	44.1	0.045	4.60
27	45.3	0.035	
30	46.7	0.035	5.20
34	48.0	0.032	
37	48.5		5.40
41	50.3		
42	49.2		
44	49.9		5.00
50	51.5		4.70
58	52.3		5.50
65	53.1		6.10

TABLE B-20

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN F-I-20 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.110	0.32
1	3.4	0.140	
2	6.2	0.187	0.37
3	8.2	0.257	
4	11.1	0.435	
5	13.7	0.737	
6	16.1	1.087	0.42*
7	22.2	1.690	
8	26.5	2.225	
9	32.3	2.525	1.02*
10	35.5	2.375	
11	38.7	1.325	
12	41.9	0.255	
13	44.1	0.050	
14	45.6	0.040	
15	46.7	0.040	
16	47.2	0.045	6.20
20	50.1	0.035	
23	51.4	0.032	5.00
27	52.1	0.032	
30	52.6	0.032	5.40
34	52.6	0.025	
36	54.4	0.032	
37	54.9		5.90
44	56.6	0.020	5.60
50	57.7	0.025	5.20
54	58.2		5.50

*Questionable accuracy because of high nitrite value

TABLE B-21

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN F-I-25 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.110	0.25
1	4.4	0.175	
2	7.6	0.285	0.35
3	10.3	0.434	
4	13.5	0.770	
5	18.0	1.212	
6	24.2	1.850	0.45*
7	24.5	2.225	
8	27.5	2.325	
9	30.1	1.850	1.47*
10	33.3	0.450	
11	36.5	0.080	
12	36.6	0.045	
13	37.7	0.040	
14	39.5	0.045	
15	39.3	0.050	
16	41.0	0.072	3.80
20	41.9	0.120	
21		0.140	
23	43.1	0.295	3.30
27	45.3	0.040	
30	48.7	0.040	3.10
34	49.4	0.045	
37	50.8	0.050	3.30
44	55.9	0.020	3.80
50	57.4	0.027	3.90

*Questionable accurace because of high nitrite value

TABLE B-22

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN F-I-30 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.125	0.25
1	6.6	0.187	
2	11.2	0.275	0.35
3	15.0	0.450	
4	20.5	0.820	
5	26.5	1.262	
6	30.0	2.125	0.47*
7	33.4	2.800	
8	37.1	3.075	
9	40.0	2.325	2.22*
10	45.9	0.375	
11	45.6	0.035	
12	48.8	0.027	
13	48.2	0.027	
14	47.1	0.027	
15	47.7	0.032	
16	48.3	0.045	5.20
20	50.5	0.257	
21		0.032	
23	52.4	0.032	5.20
27	56.1	0.027	
30	61.8	0.032	5.80
34	57.7	0.025	
36	58.4		
37	59.2	0.032	5.40
44	61.3	0.025	5.60
50	62.4	0.032	6.20

*Questionable accuracy because of high nitrite value

TABLE B-23

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN G-III-10 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.207	0.35
1	1.6	0.202	
2	3.0	0.212	
3	4.0	0.217	0.35
4	5.2	0.212	
5	6.8	0.212	
6	10.0	0.222	
7	8.7	0.227	
8	10.2	0.242	
9	10.7	0.232	
10	13.7	0.237	0.45
11	12.4	0.262	
12	12.1	0.242	
13	13.1	0.252	
14	13.6	0.280	
15	14.9	0.290	
16	15.0	0.330	
17	14.8	0.340	0.45
20	16.9	0.485	
23	19.9	0.685	0.70
27	23.0	0.937	
30	27.1	0.612	3.50
34	37.2	0.055	
37	45.3	0.050	6.60
38	44.0		
45	46.1	0.027	7.50
52	48.5	0.032	7.40
59	49.8	0.027	6.60#
68	50.6	0.040	7.80

TABLE B-24

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN G-III-15 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.207	0.35
1	2.7	0.202	
2	4.3	0.212	
3	6.3	0.217	0.42
4	7.3	0.222	
5	9.1	0.232	
6	10.3	0.247	
7	10.8	0.257	
8	12.1	0.275	
9	12.3	0.320	
10	14.7	0.360	0.47
11	15.1	0.437	
12	17.9	0.560	
13	18.1	0.710	
14	19.0	1.037	
15	21.6	1.400	
16	24.1	1.870	
17	25.0	2.325	1.20*
20	35.1	2.187	
23	43.5	0.225	5.90
27	45.5	0.035	
30	48.2	0.040	6.2
31	47.9		
34	48.6	0.040	
37	49.7	0.045	6.80
45	51.3	0.035	6.60
52	52.7	0.045	6.60
59	54.4	0.020	6.00#
68	55.5	0.035	6.60

*Questionable accuracy because of high nitrite value

TABLE B-25

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN G-III-20 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.207	0.35
1	4.0	0.207	
2	6.1	0.222	
3	8.3	0.227	0.42
4	10.3	0.232	
5	12.5	0.265	
6	13.3	0.320	
7	14.8	0.365	
8	17.2	0.515	
9	19.1	0.825	
10	22.5	1.262	0.60*
11	25.1	1.825	
12	28.7	2.800	
13	32.6	3.457	
14	36.0	3.000	
15	41.5	1.450	
16	43.6	0.275	
17	46.1	0.047	6.50
20	48.7	0.040	
23	50.1	0.047	6.20
24	50.0		
27	46.2	0.035	
30	50.2	0.032	6.40
34	50.9	0.027	
37	51.5	0.027	7.00
45	52.8	0.025	7.50

*Questionable accuracy because of high nitrite value

TABLE B-26

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN G-III-25 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/l)	Effluent NO_2-N (mg/l)	Effluent NO_3-N (mg/l)
0	--	0.207	0.35
1	4.6	0.207	
2	7.3	0.222	
3	9.7	0.230	0.37
4	12.2	0.275	
5	14.7	0.345	
6	16.7	0.780	
7	18.9	0.840	
8	19.9	1.387	
9	23.4	2.375	
10	26.9	3.300	0.67*
11	30.2	4.100	
12	34.2	3.475	
13	37.0	1.749	
14	40.0	0.537	
15	42.6	0.065	
16	41.8	0.027	
17	41.8	0.032	7.00
20	46.5	0.027	
23	48.0	0.027	6.00
24	46.9		
27	48.1	0.027	
30	49.4	0.032	6.50
34	51.1	0.025	
37	50.9	0.025	7.50
38	51.1		
45	53.2	0.025	8.00

*Questionable accuracy because of high nitrite value

TABLE B-27

BOD, NITRITE AND NITRATE ANALYSES FOR TEST RUN G-III-30 CONDUCTED ON JUG DILUTION SAMPLES

Incubation Period (Days)	Effluent BOD (mg/1)	Effluent NO_2-N (mg/1)	Effluent NO_3-N (mg/1)
0	--	0.207	0.35
1	4.8	0.212	
2	8.4	0.223	
3	11.9	0.255	0.42
4	16.2	0.320	
5	18.9	0.550	
6	22.3	0.962	
7	22.9	1.650	
8	26.3	3.000	
9	31.0	4.406	
10	33.8	4.894	0.77*
11	35.4	5.562	
12	39.4	4.850	
13	41.8	4.250	
14	41.6	2.412	
15	45.8	0.119	
16	46.8	0.025	
17	47.6	0.027	6.80
20	50.1	0.020	
23	51.0	0.025	7.00
27	52.1	0.025	
30	54.2	0.020	6.90
34	54.4	0.025	
37	55.6	0.032	7.50
38	56.2		
45	58.1	0.025	8.00

*Questionable accuracy because of high nitrite value

TABLE B-28

Nitrite Analyses for Test Run H-V-10
Conducted on Jug Dilution Samples
Using Varying Seed Quantities

Incubation Period (Days)	Effluent NO_2-N (mg/l)			
	No Seed	10 ml/l Seed*	20 ml/l Seed*	30 ml/l Seed*
0	.040	.040	.035	.040
1	.040	.045	.045	.047
2	.045	.045	.045	.047
3	.045	.045	.047	.047
4	.047	.047	.050	.050
5	.047	.047	.050	.047
6	.047	.045	.050	.047
7	.047	.045	.050	.050
8	.047	.047	.052	.052
9	.050	.047	.055	.055
10	.052	.050	.062	.060
11	.052	.052	.067	.062
12	.062	.055	.077	.077
13	.070	.062	.090	.085
14	.085	.067	.105	.090
15	.100	.072	.125	.115
16	.110	.085	.145	.127
18	.140	.105	.220	.200
19	.192	.127	.237	.217
20	.200	.135	.280	.255
21	.275	.160	.337	.310
23	.400	.250	.525	.487
24	.525	.310	.625	.575
25	.625	.375	.725	.712
26	.737	.445	.800	.875
27	.750	.531	.870	.975
30	.800	.825	.500	1.250
31	.550	.887	.220	1.200
32	.330	.912	.090	1.062
33	.132	.810	.067	.740
34	.085	.615	.055	.410
37	.027	.085	.015	.047
38	.027	.060	.015	.035

*Seed Concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

TABLE B-29

Nitrite Analyses for Test Run H-V-20 Conducted on Jug Dilution Samples Using Varying Seed Quantities

Incubation Period (Days)	Effluent NO_2-N (mg/l)			
	No Seed	10 ml/l Seed*	20 ml/l Seed*	30 ml/l Seed*
0	.040	.052	.062	.090
1	.047	.060	.062	.097
2	.050	.062	.075	.110
3	.055	.065	.080	.125
4	.072	.080	.095	.145
5	.110	.100	.115	.190
6	.210	.137	.150	.250
7	.320	.212	.227	.475
8	.525	.320	.330	.800
9	.987	.570	.590	1.287
10	1.650	1.205	1.125	2.450
11	2.250	2.125	1.975	4.125
12	4.125	3.125	3.500	5.312
13	5.000	6.750	4.875	4.875
14	4.375	4.125	5.312	2.312
15	2.125	2.250	3.750	.047
16	.085	.140	.590	.035
18	.030	.047	.040	.045
19	.040	.047	.047	.047
20	.025	.027	.027	.030
21	.025	.030	.035	.040
23	.030	.040	.045	.040
24	.027	.040	.045	.040
25	.025	.040	.040	.040
26	.012	.040	.040	.035
27	.025	.035	.035	.030
30	.020	.025	.015	.015

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

TABLE B-30

Nitrite Analyses for Test Run H-V-30
Conducted on Jug Dilution Samples
Using Varying Seed Quantities

Incubation Period (Days)	Effluent NO_2-N (mg/1)			
	No Seed	10 ml/1 Seed*	20 ml/1 Seed*	30 ml/1 Seed*
0	.040	.137	.300	.410
1	.045	.160	.370	.500
2	.062	.225	.450	.662
3	.085	.390	.640	.937
4	.132	.625	1.012	1.280
5	.252	1.400	2.025	2.385
6	.525	2.800	3.750	4.250
7	1.300	5.437	5.625	6.312
8	2.525	7.125	7.125	7.687
9	4.812	7.687	7.687	7.500
10	6.312	7.500	6.625	3.875
11	6.526	5.156	.850	.045
12	6.812	.160	.040	.030
13	6.000	.027	.035	.030
14	3.710	.030	.035	.035
15	.060	.035	.040	.040
16	.052	.030	.030	.030
18	.100	.027	.027	.030
19	.137	.025	.027	.027
20	.007	.007	.007	.007
21	.007	.007	.012	.012
23	.007	.007	.007	.007
26	.020	.002	.002	.007
30	.007	.012	.002	.002

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

TABLE B-31

Nitrate Analyses for Test Runs
H-V-10, H-V-20 and H-V-30
Conducted on Jug Dilution Samples
Using Varying Seed Quantities

Incubation Period (Days)	Effluent NO_3-N (mg/l)			
	No Seed	10 ml/l Seed*	20 ml/l Seed*	30 ml/l Seed*
Test Run H-V-10				
0	0.22	0.22	0.22	0.22
16	0.20	0.20	0.17	0.17
19	0.20	0.20	0.22	0.25
21	0.15	0.05	0.17	0.05
27	0.22	0.15	0.45	0.20
31	1.90	0.47	3.00	1.55
32	2.60	0.95	4.10	2.75
34	3.90	2.10	5.10	4.70
38	6.20	5.60	6.30	8.00
Test Run H-V-20				
0	0.22	0.22	0.22	0.22
11	0.27+	0.25+	0.27+	0.65+
15	5.50+	5.80+	4.35+	7.80
16	7.40	6.50#	7.25#	8.00#
19	7.20	8.20#	8.10#	8.30#
27	6.50	6.90	7.40	7.40
Test Run H-V-30				
0	0.22	0.22	0.22	0.22
4	0.20	0.22	0.20+	0.15+
10	0.27+	0.57+	1.65+	4.22+
12	0.35+	8.00#	8.30#	8.30#
19	7.50	8.10#	8.10#	8.60#
27	7.20	7.40	7.50	7.70

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

#Value may be too high because of problem noted with analytical procedure.

+Questionable accuracy because of high nitrite value.

TABLE B-32

Nitrite Analyses For Test Run J-VI-10 Conducted on Jug Dilution Samples Using Varying Seed Quantities and Ammonia-Nitrogen Levels

	Effluent NO_2-N (mg/1)			
Incubation Period (Days)	5 ml/1 Seed* and No NH_3-N Added	25 ml/1 Seed* and No NH_3-N Added	5 ml/1 Seed* and 5 mg/1 NH_3-N Added#	25 ml/1 Seed* and 5 mg/1 NH_3-N Added#
0	0.072	0.120	0.072	0.130
1	0.067	0.115	0.067	0.120
2	0.077	0.120	0.090	0.142
3	0.080	0.115	0.085	0.125
4	0.085	0.115	0.090	0.125
5	0.085	0.110	0.085	0.120
6	0.095	0.115	0.097	0.130
7	0.105	0.130	0.120	0.145
8	0.102	0.115	0.115	0.142
9	0.137	0.130	0.145	0.155
10	0.151	0.142	0.165	0.183
11	0.151	0.150	0.187	0.198
12	0.185	0.160	0.220	0.220
13	0.217	0.178	0.256	0.256
14	0.255	0.195	0.350	0.300
15	0.310	0.220	0.425	0.350
16	0.350	0.220	0.500	0.375
17	0.425	0.244	0.637	0.425
18	0.469	0.256	0.725	0.425
19	0.580	0.265	0.887	0.400
20	0.638	0.290	1.137	0.400
21	0.675	0.330	1.325	0.395
22	0.725	0.355	1.425	0.485
23	0.750	0.405	1.650	0.445
26	0.275	0.365	2.025	0.850
27	0.155	0.275	2.125	1.037
28	0.067	0.160	2.075	1.212
29	0.047	0.090	1.925	1.212
30	0.030	0.047	1.700	1.162
33	0.020	0.020	0.725	0.242
34	0.015	0.015	0.397	0.128

TABLE B-32

Nitrite Analyses for Test Run J-VI-10 Conducted on Jug Dilution Samples Using Varying Seed Quantities and Ammonia-Nitrogen Levels (continued)

	Effluent NO_2-N (mg/l			
Incubation Period (Days)	5 ml/l Seed* and No NH_3-N Added	25 ml/l Seed* and No NH_3-N Added	5 ml/l Seed* and 5 mg/l NH_3-N Added#	25 ml/l Seed* and 5 mg/l NH_3-N Added#
35	0.015	0.015	0.217	0.067
37	0.012	0.015	0.115	0.035
41	0.007	0.007	0.025	0.025
42	0.012	0.012	0.020	0.015

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

#NH_3-N added to diluted sample so concentration becomes 25 mg/l in terms of undiluted effluent.

TABLE B-33

Nitrite Analyses for Test Run J-VI-20 Conducted on Jug Dilution Samples Using Varying Seed Quantities and Ammonia-Nitrogen Levels

	Effluent NO_2-N (mg/l)			
Incubation Period (Days)	5 ml/l Seed* and No NH_3-N Added	25 ml/l Seed* and No NH_3-N Added	5 ml/l Seed* and 5 mg/l NH_3-N Added#	25 ml/l Seed* and 5 mg/l NH_3-N Added#
0	0.060	0.105	0.060	0.110
1	0.070	0.125	0.085	0.137
2	0.127	0.180	0.145	0.218
3	0.175	0.265	0.248	0.385
4	0.263	0.425	0.469	0.687
5	0.400	0.825	0.825	1.275
6	0.663	1.100	1.550	2.375
7	1.100	1.650	3.000	4.625
8	1.650	1.875	4.937	7.500
9	2.075	1.425	7.688	10.125
10	2.075	0.120	9.625	8.250
11	2.225	0.020	8.000	1.062
12	0.050	0.020	2.075	0.093
13	0.013	0.025	0.300	0.055
14	0.015	0.027	0.223	0.047
15	0.020	0.040	0.160	0.047
16	0.007	0.025	0.093	0.040
17	--	--	0.073	0.047
20	0.015	0.027	0.045	0.047
23	0.007	0.020	0.035	0.045
27	0.012	0.012	0.025	0.035
30	0.012	0.012	0.015	0.020
42	0.007	0.007	0.025	0.035

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

#NH_3-N added to diluted sample so concentration becomes 25 mg/l in terms of undiluted effluent.

TABLE B-34

Nitrite Analyses for Test Run J-VI-30 Conducted on Jar Dilution Samples Using Varying Seed Quantities and Ammonia-Nitrogen Levels

	Effluent NO_2-N (mg/l)			
Incubation Period (Days)	5 ml/l Seed* and No NH_3-N Added	25 ml/l Seed* and No NH_3-N Added	5 ml/l Seed* and 5 mg/l NH_3-N Added#	25 ml/l Seed* and 5 mg/l NH_3-N Added#
0	0.080	0.175	0.085	0.155
2	0.110	0.170	0.140	0.200
2	0.195	0.255	0.330	0.400
3	0.310	0.405	0.660	0.775
4	0.531	0.688	1.450	1.475
5	0.925	1.037	3.200	2.750
6	1.550	1.425	6.750	3.750
7	2.250	1.687	8.250	1.250
8	2.250	0.675	0.725	0.495
9	1.063	0.080	0.590	0.795
10	0.050	0.025	0.240	0.590
11	0.015	0.015	0.125	0.350
12	0.007	0.007	0.100	0.193
13	0.007	0.007	0.097	0.137
14	0.007	0.007	0.100	0.110
15	0.015	0.007	0.100	0.085
16	0.010	0.0	0.095	0.055
17	--	--	0.100	0.053
20	0.075	0.007	0.095	0.030
23	0.080	0.005	0.137	0.015
27	0.007	0.007	1.213	0.025
28	--	--	1.475	--
29	--	--	0.375	--
30	0.007	0.025	0.005	0.218
42	0.007	0.007	0.005	0.008

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

#NH_3-N added to diluted sample so concentration becomes 25 mg/l in terms of undiluted effluent.

TABLE B-35

Nitrate Analyses for Test Runs J-VI-10, J-VI-20 and J-VI-30 Conducted on Jug Dilution Samples Using Varying Seed Quantities and Ammonia-Nitrogen Levels

Incubation Period (Days)	Effluent NO_3-N (mg/l) 5 ml/l Seed* and No NH_3-N Added	 25 ml/l Seed* and No NH_3-N Added	 5 ml/l Seed* and 5 mg/l NH_3-N Added#	 25 ml/l Seed* and 5 mg/l NH_3-N Added#
TEST RUN J-VI-10				
0	0.20	0.45	0.17	0.45
2	0.25	0.20	0.20	0.22
5	0.17	0.23	0.13	0.20
7	0.20	0.23	0.23	0.25
9	0.23	0.30	0.23	0.25
16	0.43	0.50	0.43	0.58
23	1.60	1.85	2.00	4.50
30	4.00	4.30	14.00	23.20
42	4.60	5.10	31.50	32.00
TEST RUN J-VI-20				
0	0.20	0.45	0.17	0.45
2	0.17	0.43	0.25	0.48
5	0.22	0.58	0.25	0.68+
7	0.50+	1.00+	0.58+	1.70+
9	0.65+	2.80	3.00+	8.00+
10	--	--	--	20.00
14	3.80	4.20	28.50	32.00
16	4.00	4.60	32.00	33.00
23	4.20	4.60	31.50	33.00
30	4.20	4.60	31.00	34.00
42	4.00	4.50	31.00	32.00

TABLE B-35

Nitrate Analyses for Test Runs J-VI-10, J-VI-20 and J-VI-30 Conducted on Jug Dilution Samples Using Varying Test Quantities and Ammonia-Nitrogen Levels (continued)

	Effluent NO_3-N (mg/l)			
Incubation Period (Days)	5 ml/l Seed* and No NH_3-N Added	25 ml/l Seed* and No NH_3-N Added	5 ml/l Seed* and 5 mg/l NH_3-N Added#	25 ml/l Seed* and 5 mg/l NH_3-N Added#
TEST RUN J-VI-30				
0	0.20	0.45	0.17	0.45
2	0.37	0.48	0.22	0.55
5	0.43	0.70+	0.45+	1.18+
7	0.47+	1.50+	6.00+	10.00
9	2.00	3.60	28.00	20.00
10	--	--	29.00	20.00
14	3.10	3.80	27.00	23.50
16	3.10	3.70	29.50	25.00
23	4.10	4.10	29.00	25.00
30	4.60	3.80	32.00	24.00
42	4.50	4.40	32.00	33.00

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

#NH_3-N added to diluted sample so concentration becomes 25 mg/l in terms of undiluted effluent.

+Questionable accuracy because of high nitrite value.

TABLE B-36

Ammonia Analyses for Test Runs J-VI-10, J-VI-20 and J-VI-30 Conducted on Jug Dilution Samples Using Varying Seed Quantities and Ammonia-Nitrogen Levels

	Effluent NH_3-N (mg/1)			
Incubation Period (Days)	5 ml/1 Seed* and No NH_3-N Added	25 ml/1 Seed* and No NH_3-N Added	5 ml/1 Seed* and 5 mg/1 NH_3-N Added#	25 ml/1 Seed* and 5 mg/1 NH_3-N Added#
TEST RUN J-VI-10				
2	1.45	1.15	21.65	24.25
5	0.58	0	21.95	22.25
7	0.29	1.15	23.30	23.85
9	0	0	20.10	21.85
16	0	0	23.30	21.25
23	0	0	21.45	18.50
TEST RUN J-VI-20				
2	0.58	1.45	23.40	21.95
5	0	0	14.65	22.30
7	2.05	0	20.10	18.95
9	0	0	11.65	4.95
16	0.29	0	0	0
TEST RUN J-VI-30				
2	1.17	0	20.45	22.80
5	0	0	19.30	18.70
7	0	1.75	11.95	14.55
9	0	1.15	2.35	10.50
16	0	0	0	3.50

*Seed concentrated by centrifugation prior to use--stated quantity is the equivalent amount prior to centrifugation.

#NH_3-N added to diluted sample so concentration becomes 25 mg/1 in terms of undiluted effluent.